The role of Human Papillomavirus types 16/18 and Late-Early oncoproteins in the detection of Cervical Cancer among adult Black South African women: Evidence from the Johannesburg Cancer Study.

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Mwiza Gideon Singini

A thesis submitted to the Faculty of Health Sciences, University of Witwatersrand, in fulfilment of the requirements for the degree

of

Doctor of Philosophy in Public Health

(Cancer Epidemiology and Biostatistics)

November 2022

JOHANNESBURG, SOUTH AFRICA

Declaration

I, Mwiza Gideon Singini, declare this thesis is my original, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other University or institution.

Signed  Date: 03 November 2022

Dedication

To God, the Almighty, I have managed to get this far in life through his merciful love. To all the women out there who are currently living in pain because of cervical cancer. To my late supervisor Dr Elvira Singh (Thank you so much for your support for the 3 years we worked together). My late uncle Pajuzi Gideon Singini passed on due to throat cancer. To my late sister Chimwemwe Catherine Singini, my dear sister, how I wish you lived up to this day so you could have witnessed how far I have reached in life.

Conference Proceedings

Oral: Singini MG, Singh E, Bradshaw D, Chen W.C, Motlhale M, Kamiza AB, de Villiers CB, Muchengeti M, Mathew CG, Newton R, Bender N, Waterboer T and Sitas F (2022). HPV types 16/18 L1 E6 and E7 proteins seropositivity and cervical cancer risk in HIV-positive and HIV-negative Black South African women. ICGBEB, Date: 16-18 August 2022, Harare, Zimbabwe.

Poster: Singini MG, Singh E, Bradshaw D, Chen W.C, Motlhale M, Kamiza AB, de Villiers CB, Muchengeti M, Mathew CG, Newton R, Bender N, Waterboer T and Sitas F (2022). HPV types 16/18 L1 E6 and E7 proteins seropositivity and cervical cancer risk in HIV-positive and HIV-negative Black South African women. ICGBEB, Date: 16-18 August 2022, Harare, Zimbabwe.

International workshops attended

International Centre for Genetic Engineering and Biotechnology (ICGBEB)

Title: Human papillomavirus (HPV) and related cancers; Prevention,

Screening, Diagnosis and Priority research areas for LMICs Date: 16-18 August 2022. Venue: The Bronte Garden Hotel, 132 Baines Avenue, Harare, Zimbabwe

Original papers

1. Singini MG, Singh E, Bradshaw D, Chen WC, Motlhale M, Kamiza AB, de Villiers, CB, Muchengeti M, Mathew CG, Newton R, Bender N, Waterboer T and Sitas F (2022). Usefulness of high-risk HPV early oncoprotein (E6 and E7) serological markers in the detection of cervical cancer: A systematic review and meta-analysis. J Med Virol 1-10.

2. Singini MG, Singh E, Bradshaw D, Chen W.C, Motlhale M, Kamiza AB, de Villiers CB, Muchengeti M, Mathew CG, Newton R, Bender N, Waterboer T and Sitas F (2022). HPV types 16/18 L1 E6 and E7 proteins seropositivity and cervical cancer risk in HIV-positive and HIV-negative Black South African women. Infectious Agents Cancer 17, 14.

3. Singini MG, Sitas F, Bradshaw D, Chen WC, Motlhale M, Kamiza AB, de Villiers C.B, Lewis C.M, Mathew CG, Waterboer T, Newton R, Muchengeti M, Singh E (2021) Ranking lifestyle risk factors for cervical cancer among Black women: A case-control study from Johannesburg, South Africa. PLoS ONE 16(12): e0260319.

Other publications within the PhD period

1.0 Kamiza AB, Kamiza S, Singini MG, Mathew CG. Association of TP53 rs1042522 with cervical cancer in the sub-Saharan African population: a meta-analysis. Trop Med Int Health. 2020 Jun;25(6):666-672.

2.0 Motlhale M, Sitas F, Bradshaw D, Chen WC, Singini MG, de Villiers CB, Lewis CM, Muchengeti M, Waterboer T, Mathew CG, Newton R, Singh E. Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult Black South Africans: A case-control study, Cancer Epidemiology 78, 2022: 102158.

3.0 Kamiza AB, Fatuma S, Singini MG, Yeh CC and Chikowore T (2022). Hepatitis B infection is causally associated with extrahepatic cancers: A Mendelian randomisation study. eBioMedicine 2022;79: 104003

4.0 Motlhale M, Sitas F, Bradshaw D, Chen WC, Singini MG, de Villiers CB, Lewis CM, Muchengeti M, Waterboer T, Mathew CG, Newton R, Singh E. Epidemiology of Ka posi's sarcoma in sub-Saharan Africa, Cancer Epidemiology, 78, 2022: 10216

Abstract

Cervical cancer screening programmes using Papanicolaou (Pap) testing or human papillomavirus (HPV) detection from cervical smears and other invasive biopsy-based assays have been met with limited success in South Africa, which indicates a need to explore other less invasive options for screening. One proposed option is serology testing for overexpression of high-risk human papillomavirus (hrHPV) early 6 or 7 (E6/E7) oncoproteins. The overexpression of E6/E7 oncoproteins corresponds with serological data, showing strong associations with cervical cancer. In conjunction with other suspected infections such as Human immunodeficiency virus (HIV) and other co-factors, these may allow clinicians or screening programmes to identify women who are at high risk of cancerous lesions of the cervix earlier than currently identified. However, in South Africa, data from large lifestyle and serological-based cancer studies in HIV-positive and negative individuals are lacking. This PhD assessed the importance of the HPV-16 and -18 late and early oncoproteins in detecting cervical cancer among adult Black South African women using data from the Johannesburg Cancer Study (JCS). The PhD is made up of three main components of work:

Firstly, we conducted a systematic review and meta-analysis to investigate HPV-antibodies' utility, especially of hrHPV-16/18 E6 and E7 serological markers, in detecting cervical cancer and cervical intraepithelial neoplasia (CIN) 2/3. We identified three articles from 69 reports, all showing low sensitivity but high specificity for detecting cervical cancer. One study used Enzyme-linked Immunosorbent Assay (ELISA), another used a Multiplex immunofluorescent assay, and the last used an immunoenzymatic assay (Slot Blot) to identify HPV seropositivity. Our finding showed that HPV-16/18 E6 and E7 serological markers have high specificity, but sensitivity is suboptimal for detecting cervical cancer. This study was published in the Journal of Medical Virology in 2022.

Secondly, using data from the JCS, a case-control analysis study was conducted. We assessed HPV types 16/18 L1 E6 and E7 oncoprotein seropositivity and their relation to cervical cancer risk in HIV-positive and HIV-negative Black South African women. In addition, assessment of the utility of HPV-16 and -18 E6 and E7 antibodies in identifying cases of cervical cancer. This was carried out based on the gap identified in the systematic review. The findings from this study showed that HPV-16 E6 and E7 antibody positivity was positively associated with cervical cancer in HIV-positive (Adjusted Odds Ratio (AOR)=33;95% CI:10-107) and HIV-negative women (AOR=97;95% CI:46-203). In HIV-positive women, HPV E6/E7 antibodies had low sensitivity (43.0%) and high specificity (90.6%) for cervical cancer detection. In HIV-negative women, HPV E6/E7 antibodies sensitivity was 70.6%, and specificity was 89.7%. By comparison, conventional Pap smears have a sensitivity of 54% and specificity of 97% in detecting cervical atypical squamous cells of undetermined significance (ASCUS) or worse. The findings from this study would help inform policymakers on the importance of HPV E6 antibodies as a future test for detecting cervical cancer among HIV-positive and HIV-negative women. The study was published in Infectious Agents and Cancer in 2022.

Thirdly, we ranked lifestyle risk factors for cervical cancer among Black women in an urban setting. In decreasing order of priority, cervical cancer was associated with (1) being HIV positive, (2) having lower educational attainment, (3) having higher parity, (4) hormonal contraceptive use, (5) heavy alcohol consumption, (6) current smoking and (7) rural residence (noting, the JCS was representative of an urban setting. If we repeated the JCS in a rural setting the rankings would come out differently). These findings could inform medical systems on key risk factors to integrate into cervical cancer education literacy programmes. In addition, these findings would help healthcare personnel involved in sensitising women on cervical cancer screening to understand the public-health gains that result from minimising the risk of each factor. The study was published in PLoS One in 2021.

Overall, this PhD research has demonstrated that HPV E6 or E7 antibodies may have a novel role as serological markers for detecting cervical cancer among Black women. Furthermore, a screening strategy that focuses on women at the highest risk (by ranking known cervical cancer co-factors in an urban setting) can be applied as a point-of-care or screening test to address the problem of low turn-out due to the current methods for cervical cancer screening. The critical contribution of this PhD includes: identifying a screening tool that could be used in a place with limited infrastructures and gynaecologists to screen women for cervical cancer. In addition, using the largest dataset in Africa, it has provided the most up-to-date and accurate findings on the accuracy of serological screening for cervical cancer. Furthermore, this PhD identified that HPV-16/18 early oncoprotein markers are less sensitive in HIV-positive women, suggesting other HPV types other than 16 and -18 may be co-responsible. In addition, it identified and ranked cervical cancer risk factors in order of importance in an urban setting. Overall, the findings from this PhD are essential in contributing to cervical cancer prevention and elimination strategies.

Acknowledgements

I am sincerely thankful to my supervisors' Associate Professor Freddy Sitas, Dr Mazvita Muchengeti and the late Dr Elvira Singh, for their constant support and insightful guidance throughout the research process. I am also grateful to my supervisors for believing in me and that I could do this work.

I am grateful to the South African Medical Research Council (with funds received from the South African National Department of Health) and the UK Medical Research Council (with funds from the UK Government's Newton Fund) (MRC-RFA-SHIP 01- 2015) for funding this study under the project evolving risk factors for cancer in African populations (ERICA-SA) and Wits Postgraduate Merit Award for the financial support throughout my tenure as a PhD student. This work would not have been possible if it were not for these two bursaries I received. Despite other far deserving students than myself but I still received the bursaries.

My heartfelt thanks to my parents (Helms John Alex Singini and Susan Nyirenda Singini) and my siblings for their prayers and support throughout this journey. Your support for me was immense during my PhD journey. I remember how I battled with life due to COVID-19 during the second year of my PhD journey, but you never gave up on me.

My sincere gratitude to Professor Tobias Chirwa, Professor Jonathan Levin, Dr Felix Chilunga, Dr Mitch Matoga, Mr Steady Chasimpha, Dr Noel Patson, Dr Augustine Choko, Dr Howard Dominic Banda, Dr Abram Bunya Kamiza, Dr Richard Munthali, Dr Okechinyere Achilonu, Dr Melitah Mothla, Dr Albert Shara, and PhD students at Wits School of Public Health, Staff at South African National Cancer Registry, The PhD coordinator, School of Public Health at WITS University and all my friends who gave me the moral support during this Journey.

Lastly, I am thankful to my wife, Taonga Martha Njikho. You have been supportive of my choice to do my PhD studies, despite being fully aware that the PhD journey would bring a long-distance relationship between us. Taonga, I did this for us, for our future and above all, for the greater glory of God.

Preface

"Eliminating any cancer would have once seemed an impossible dream, but we now have the cost-effective, evidence-based tools to make that dream a reality. However, this can be achieved if we match the power of the tools we have with unrelenting determination to scale up their use globally." Dr Tedros Adhanom Ghebreyesus (WHO Director-General), 2020.

In low-and middle-income countries, efforts to reduce cervical cancer incidence have been met with limited success. This is because the current methods for screening for cervical cancer in these settings require significant infrastructure and are relatively invasive, such that incidence rates for this disease remain high.

After completing my Master's degree in Demography and Population Studies at the University of Witwatersrand in 2017, where I researched the risk factors of obstetric fistula in Zambia, I left South Africa for Malawi. In 2018, I started working as a Senior Monitoring and Evaluation Officer at Thyolo district hospital in Thyolo, Malawi, under a project called Data for Action by the KUUNIKA organisation. While working in Thyolo, I had a chance to visit health facilities in the rural areas of the district. One of the areas I saw was the HIV clinic. From these visits, I learned from the medical assistants that aside from the high burden of HIV in the district, cervical cancer was also a problem of great concern, mainly because of the low uptake of cervical cancer screening due to poor accessibility. It was against this background that I got interested in studying novel approaches to cervical cancer screening. Hence, responding to the global call for cervical cancer elimination by WHO.

In the same year, the South African Medical Research Council, under the project Evolving Risk Factors for Cancers in African populations (ERICA-SA), advertised PhD positions to study Cancer Epidemiology. I applied for the post, and I was one of the successful candidates. I then got introduced to the area of serological markers by my supervisors, the late Dr Elvira Singh and Associate Professor Freddy Sitas, and the rest of the ERICA-SA team, comprising Professors Debbie Bradshaw, Chris Mathew, Rob Newton, Cathryn Lewis and Dr Tim Waterboer. Through their guidance and mentorship, I was challenged to research a hypothesis proving the utility of "serological markers for HPV-16 and -18 late-early proteins as useful markers for the detection of cervical cancer."

Structure of the thesis

This thesis is composed of 3 parts. Part one consists of chapters one and chapter two. Part two is the empirical findings (Chapters three to five), and Part three is the general discussion (Chapter six) and Conclusions (Chapter seven). Thus, this PhD thesis is by publication and comprised of three papers published in different international journals and seven chapters. The three papers form the main chapters (Chapters three, four and five) of the PhD thesis and an introduction for each chapter.

Chapter one of the thesis provides the background, problem statement and significance of the study, literature review, conceptual framework, research question, study aim and specific objectives where necessary figures have been used to show the burden of the disease and description of the HPV genome.

Chapter two of the thesis provides a summary of the methodology. This chapter provides the study design and setting, study population, definition of cases and controls, statistical analysis, my role as a PhD student, collaborations, ethical consideration and funding.

Chapter three is the systematic review and meta-analysis that was conducted of diagnostic test accuracy studies where it investigated the usefulness of hrHPV early oncoprotein (E6 and E7) serological markers in the detection of cervical cancer. This work was carried out to assess the utility of HPV antibodies as a screening test (in terms of sensitivity and specificity). This work was published in the Journal of Medical Virology Journal.

Chapter four reports the association between HPV types 16/18 L1 E6 and E7 proteins seropositivity and Cervical Cancer Risk in HIV-Positive and HIV-Negative Black South African Women. This paper was published in Infectious Agents Cancer in 2022.

Chapter five presents the findings of this PhD on "Ranking lifestyle risk factors for cervical cancer among Black women. A case-control study from Johannesburg, South Africa". This work was published in PLoS One in 2021.

Chapter six is the general discussion for the PhD thesis. The discussion section provides an opportunity to assess the extent to which this PhD answered the objectives, validation of the conceptual framework and reflected on the emerging themes. It also discusses the significant limitations and strengths of the study and the implications of the findings for public health. The chapter ends with recommendations for future work, policy implications and recommendations. Chapter seven is the Conclusion of the thesis.

Author contribution

I was the lead on all of the work included in this thesis. I conducted a literature search and a systematic review, developed a protocol, applied for ethics clearance, and worked on the information from the JCS. I conceptualised the overall method to address the research questions before looking for input from my supervisors. My supervisors then advised me on the appropriate collaborators to include for specific project components. For example, I contacted Dr Tim Waterboer to collaborate on the serology laboratory methods. I wrote and revised the research proposal and obtained ethics approvals. I cleaned and analysed the data, wrote the first drafts of all the manuscripts, collated comments from the other authors, and was responsible for revisions and final journal submissions.

Collaborations

Current collaborations from the ERICA-SA project that I utilised in the course of this project were the following colleagues and their institutions:

a. The late Dr Elivra Singh

    i. South African National Cancer Registry, National Health Laboratory Service

    ii. Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

b. Associate Professor Freddy Sitas

    i. Centre for Primary Health Care and Equity, School of Population Health, University of New South Wales Sydney, Australia

    ii. Menzies Centre of Health Policy, School of Public Health, University of Sydney, Australia

    iii. Burden of Disease Research Unit, South African Medical Research Council

c. Professor Debbie Bradshaw

    i. Burden of Disease Research Unit, South African Medical Research Council

d. Professor Christopher G. Mathew

    i. Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

    ii. Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College London, SE1 9RT, United Kingdom

e. Professor Robert Newton

    i. MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda

    ii. University of York, York, United Kingdom

f. Professor Cathryn M. Lewis

    i. Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, SE5 8AF, United Kingdom

    ii. Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College London, SE1 9RT, United Kingdom

g. Dr Tim Waterboer and Dr Noemi Bender

    i. Division of Infections and Cancer Epidemiology, German Cancer Research Centre (DKFZ), Heidelberg, Germany

h. Dr Chantal Babb de Villiers

i. Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

ii. PHG Foundation, University of Cambridge, United Kingdom

i. Dr Mazvita Muchengeti

i. South African National Cancer Registry, National Health Laboratory Service

ii. Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

iii. South African DSI-NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), Stellenbosch University, Stellenbosch, South Africa

j. Dr Wenlong Carl Chen

i. South African National Cancer Registry, National Health Laboratory Service

ii. Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

k. Dr. Abram Bunya Kamiza

i. Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

l. Dr Melitah Motlhale

i. South African National Cancer Registry, National Health Laboratory Service

ii. Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

m. Mr Thendo Ramaliba

    i. Burden of Disease Research Unit, South African Medical Research Council

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List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AOR	Adjusted Odds Ratio
ASCUS	Atypical Squamous Cells of Undetermined Significance
AUC	Area Under the Curve
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
DFKZ	Deutsches Krebsforschungszentrum (German Cancer Research Centre)
DNA	Deoxyribonucleic Acid
E6/E7	Early Oncoproteins 6/7
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food And Drug Administration
FN	False Negative
FP	False Positive
GST	Glutathione S-Transferase
HAART	Highly Active Antiretroviral Therapy
HIC	High-Income Countries
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
hrHPV	High-Risk Human Papillomavirus
HSIL	High-Grade Squamous Intraepithelial Lesion
HSROC	Hierarchical Summary Receiver Operating Characteristics
ICC	Invasive Cervical Cancer
ICD-O	International Classification of the Diseases for Oncology
IgG	Immunoglobulin

IQR	Interquartile Range
JCS	Johannesburg Cancer Study
L1	Major Capsid Protein Late 1
L2	Minor Capsid Protein Late 2
LCR	Long Control Region
LMIC	Low-and Middle-Income Countries
LSIL	Low-Grade Squamous Intraepithelial Lesion
MFI	Median Fluorescence Intensity
mRNA	Messenger Ribonucleic Acid
PAP	Papanicolaou
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PCR	Polymerase Chain Reaction
pRb	Retinoblastoma
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies-2
ROC	Receiver operating characteristic
SSA	Sub-Saharan Africa
SST	Serum Separation Tube
TCT	Thinprep Cytological Test
TN	True Negative
TP	True Positive
USA	United States of America
VIA	Visual Inspection-Acetic Acid
VIP	Visual Inflection Point
WHO	World Health Organization

PART 1
INTRODUCTION AND BACKGROUND
LITERATURE REVIEW
METHODOLOGY

1.0. Chapter One: Introduction and Background

1.1. Introduction

This chapter provides the background of the study, a statement of the problem and the significance of the study. It also gives a detailed description of the human papillomavirus genome, which consists of early and late proteins and the upstream regulatory region (URR). The chapter further discusses the literature on HPV detection, seroepidemiology of HPV-16 and -18 antibodies, lifestyle risk factors for cervical cancer and a conceptual framework for this study. The chapter highlights the research question, aim and specific objectives.

1.2. Background

Cervical cancer constitutes a public health problem globally. It is the fourth most occurring incident cancer and the fourth leading cause of cancer-related deaths in women (Figure 1-1) [1]. In 2020, approximately 604,127 incident cases (Figure 1-2) and 341,831 cervical cancer deaths occurred worldwide [1].

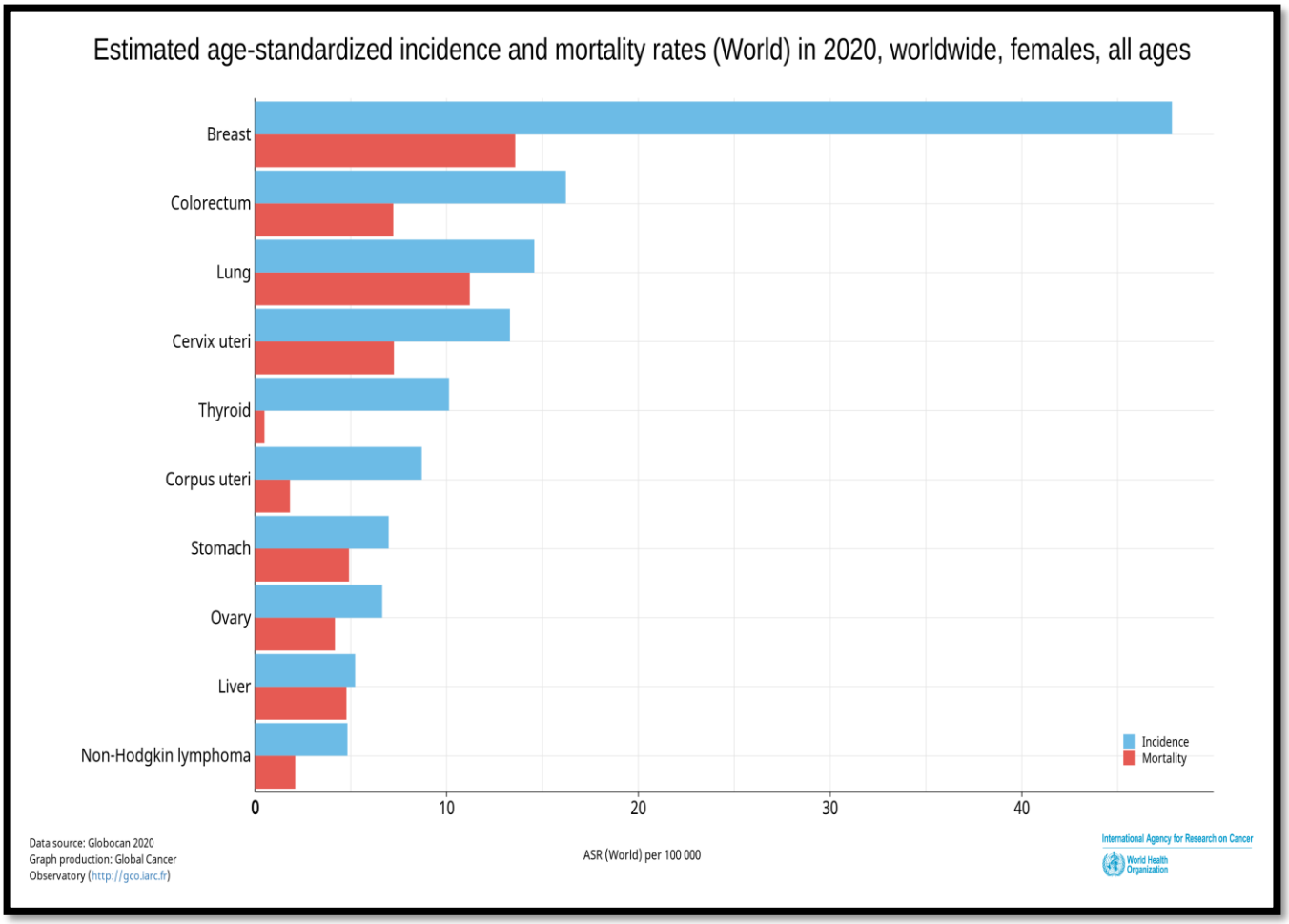


Figure 1-1: Estimated age-standardised incidence and mortality rates for the top 10 cancers: reproduced with permission from International Agency for Research on Cancer (IARC). Source: Global cancer, incidence, mortality and prevalence (GLOBOCAN) [2].

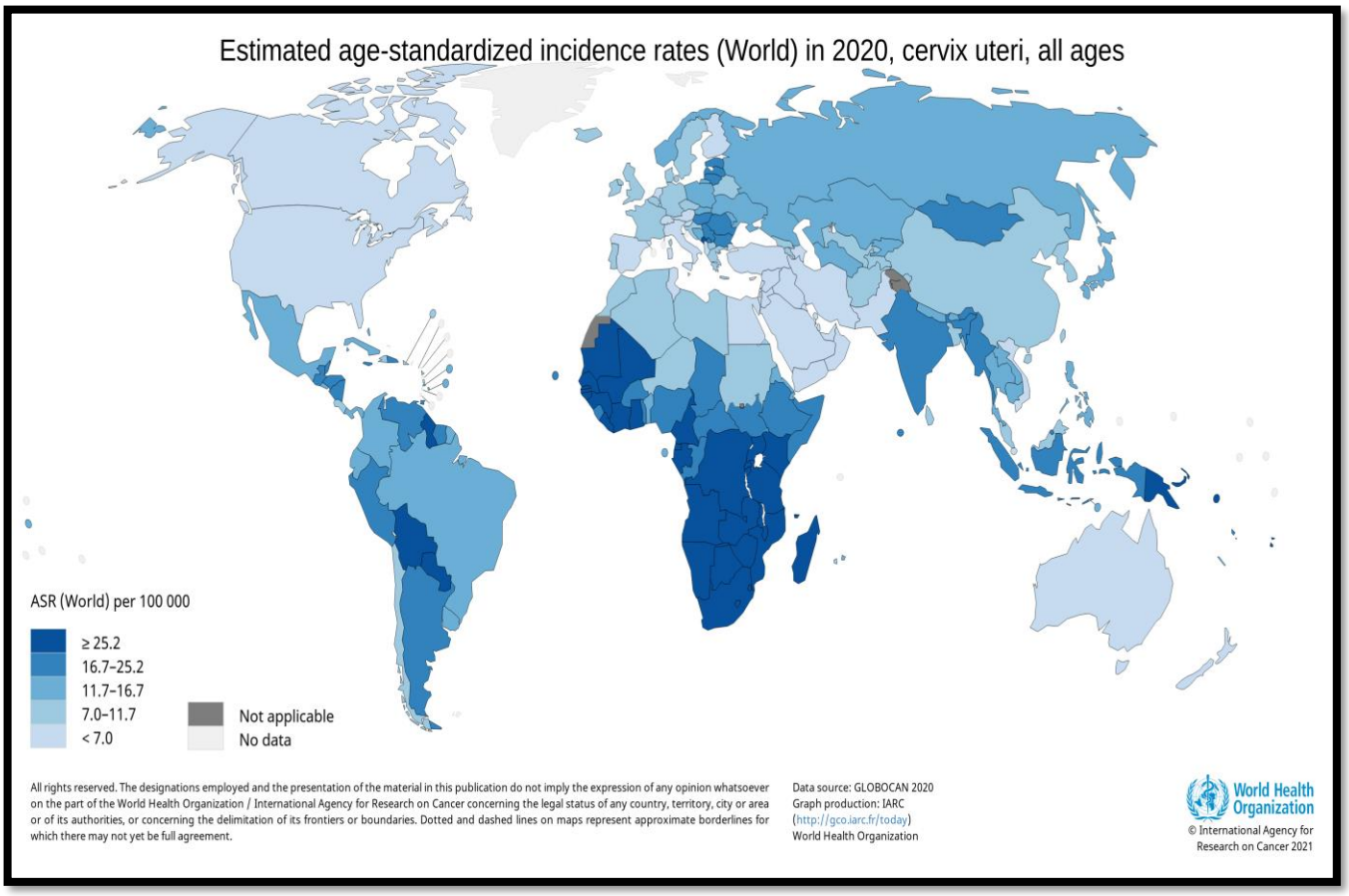


Figure 1-2: Estimated incidence of cervical cancer globally in 2020. Reproduced with permission from IARC Source GLOBOCAN [2].

The majority of incident cervical cancers (84%) and deaths (88%) are from low- and middle-income countries (LMICs) (Figures 1-3 and 1-4) [3]. In the same year, according to GLOBOCAN, in Southern Africa, the age-standardised incidence rate was 35.4 per 100,000 women (Figure 1-4), and the age-standardised mortality was 20.6 per 100,000 women [1].

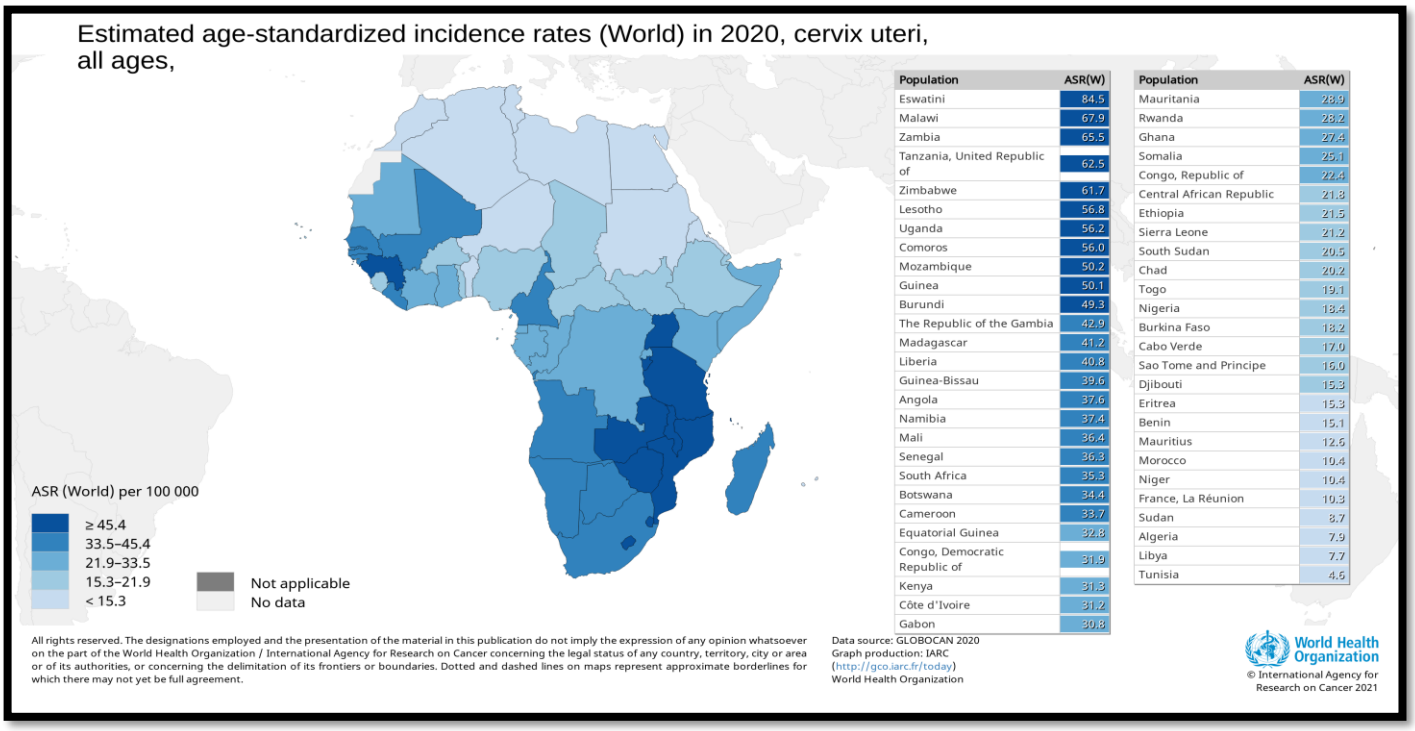


Figure 1-3: Estimated incidence of cervical cancer in Africa. Reproduced with permission from IARC Source GLOBOCAN [2].

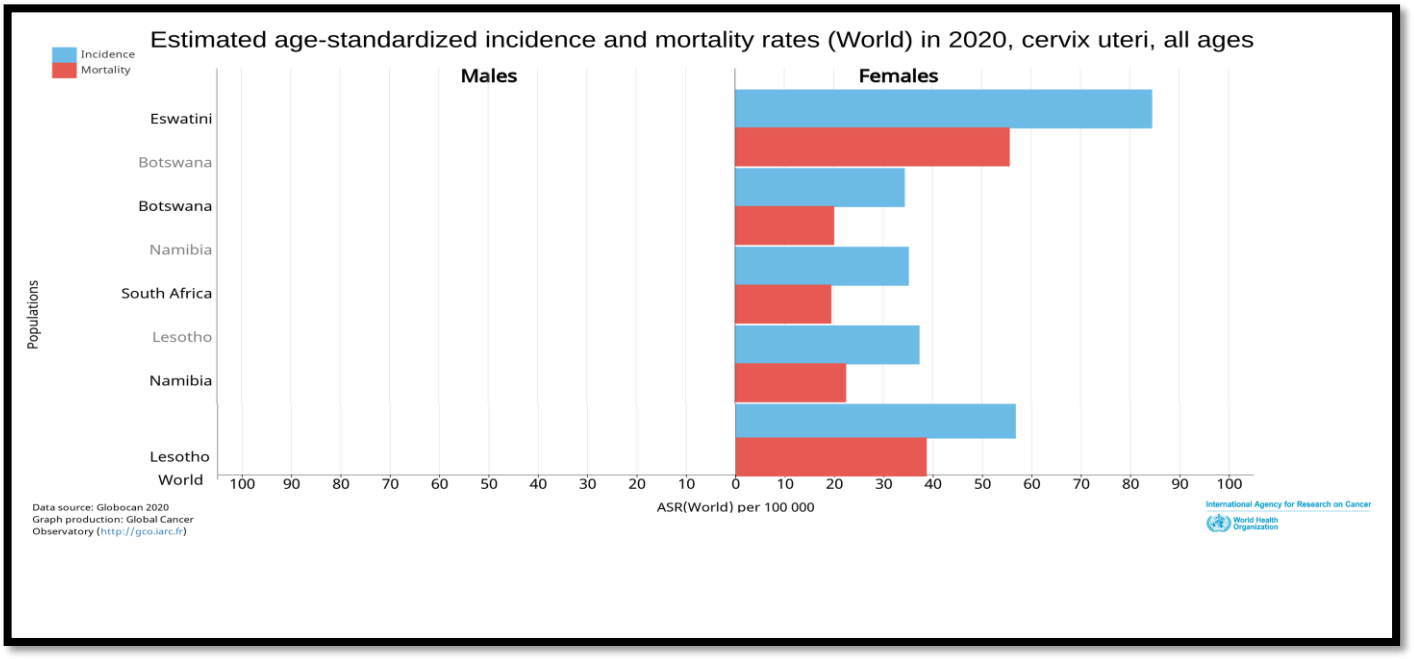


Figure 1-4: Estimated age-standardized incidence and mortality rates in Southern African countries in 2020. Reproduced with permission from IARC Source: GLOBOCAN [2].

High-risk Human papillomaviruses (hrHPV) are responsible for almost all cervical cancers [4]. Prophylactic HPV vaccines offer a primary prevention method for at least 80% of cervical cancers in LMICs [5,6]. However, about 3.7 billion older teenagers and adult women worldwide (1 876 million teenagers and adult women) in LMICs [3] with HPVs infection [7] have missed the opportunity for HPV vaccination. They risk developing cervical cancer in the next 30-40 years [8–10].

Despite efforts to screen for cervical cancer using Papanicolaou (Pap) or HPV-based cervical smears in South Africa, screening coverage is low, estimated at 46.8% [11]. Factors attributing to the low screening coverage in South Africa include poverty, limited resources and infrastructure in rural settings. In rural South Africa, most health centres lack formal private rooms where women can privately self-collect samples. In addition, some clinics lack examination beds, have a shortage of qualified healthcare workers, and have limited functional public laboratories to conduct the test [12]. Women lack decisions making power to attend cervical cancer screening [13]. The nurses who do Pap smears are the same ones required to see general patients at clinics. Furthermore, they have long queues of sick patients. They are unlikely to do smears on asymptomatic women due to a shortage of specula [14]. Therefore, the incidence of cervical cancer remains high, and detection is often at a late stage [15]. In addition, a high burden of Human immunodeficiency virus (HIV) in South Africa contributes to increased disease incidence. HIV-positive women have a high incidence rate of pre-cancerous and invasive cervical cancer [15]. Moreover, persistent infections with HPV-16 and -18 are responsible for most cervical cancers [16]. Both are targeted by the nationwide (bivalent -16 and -18) HPV vaccination program for girls 9 to 10 years, conducted by the South African Department of Health [17]. Thus, efforts to improve secondary prevention rates (screening) of cervical cancer, including in LMICs, are still imperative. As conventional screening methods have failed in Africa to reach acceptable coverage rates (>70%) as recommended by the World Health Organisation (WHO) [18]. Hence, a more acceptable and effective method that may be more accessible to women is required. Although the South African government has established a

cervical cancer screening policy in the public sector to screen 70% of women at the ages of 30,40, and 50; however, with the current low screening rates and follow-up, these goals have not been achieved [19,20].

An innovative way of addressing the low HPV screening rates in South Africa could be using less invasive serological biomarkers. After HPV infection, antibodies to HPV early (E6 and E7) proteins are induced in the host's serum. These markers may be potential disease markers because their seroprevalence increases with the severity of the tumour [21–24].

The presence of hrHPV DNA in the cervix is a marker of new HPV infections [25], while the presence of HPV L1 in serum is a marker of past infections [26]. The overexpression of HPV E6/7 oncoproteins indicates HPV-driven oncogenesis and, thus, is a potential marker of a higher risk for cervical cancer [27]. Hence, the data generated from this analysis provides evidence for policymakers to consider the feasibility of implementing or reviewing cervical cancer screening programmes.

Over the years (since 1960), in high-income countries (HICs), the implementation of population-based screening of cervical cancer has led to a substantial decline in this disease and mortality [28]. However, such public health benefits have not yet been observed over the same period in LMICs like South Africa [29]. Some of the reasons contributing to the failure of the cervical cancer screening programme in LMICs include low coverage, poor access to health facilities, lack of knowledge of the disease among women, lack of resources on cervical cancer screening methods and a high burden of HIV and acquired immune deficiency syndrome (AIDS). Currently, there are no less-invasive screening biomarkers for detecting cervical cancer at an earlier stage [8]. The advantages of less invasive serological biomarkers

over traditional screening methods are that they are quick, cheap, and simple to use by women. They require little blood routine collection from participants [30].

Empirical evidence suggests that HPV-16 and -18 (E6 and E7) serology are promising biomarkers for detecting cervical cancer [30]. For example, findings from HICs have shown high odds ratios for cervical cancer (OR= 64.4, 95% CI: 36 -137) [31] in Caucasian populations. These data are essential as they present possibilities for identifying less-invasive biomarkers that would offer a window of opportunity to identify individuals at higher risk of the disease [8,32]. However, such data are still lacking in low-income countries, specifically Black African populations, where cervical cancer and HIV infections are high. This problem is also exacerbated by the lack of access to screening, therefore, limited tissue-based HPV DNA [30].

In South Africa, the Johannesburg Cancer Study (JCS) [33] was established in 1995 to redress the historical lack of epidemiological cancer research among Black South Africans at that time. In the South African context, the word Black refers to individuals with African, Indian and Coloured backgrounds [34]. In the JCS, Black refers to individuals with an African background. The JCS aimed to examine whether key known and emerging risk factors for cancer in Western countries (i.e. mainly Caucasian populations) applied to Black patients locally in the urban setting of Johannesburg, South Africa. These aims evolved into measuring the relative importance of known and emerging risk factors for cancer in a local setting and establishing a biobank and the largest study (n=26,000) on the continent for ongoing investigations of lifestyle, infectious and genetic drivers of cancer.

Only one previous serological case-control study on the JCS focused on HPV-16 and HPV-related cancers [35]. The study focused on anti-HPV-16 (L1) immunoglobulin (IgG) antibody levels using an enzyme-linked immunosorbent assay (ELISA). More recently, the JCS sera were tested for various HPV antibodies (16, 18, L1, E6, E7) using a high throughput multiplex immunofluorescent assay technique. Therefore, this provided a unique opportunity to use the newly available serology data from the JCS to explore whether HPV-16 and -18 (L1, E6 and E7) proteins may be used as HPV-related tumour biomarkers for earlier diagnosis of cervical cancer in South Africa. In addition, previous studies have shown that cervical cancers and HIV are more common among Black populations than in other populations [15,29,36]. In light of this situation, this study focused on the adult Black population of South Africa. A validated multiplex serology testing approach was used to assess HPV-16 and -18 antibodies based on a glutathione S-transferase (GST) immunosorbent assay (used in several settings and other large studies) [37].

1.3. Statement of the Problem

In South Africa, the incidence rate of cervical cancer is high. Among Black South African women, HIV contributes to the high incidence rate of cervical cancer [38]. Reducing cervical cancer through HPV vaccination, early detection, and screening is one of the goals of the South African national cancer prevention policy. However, screening programs using Pap testing or HPV detection from cervical smears and other invasive biopsy-based assays have been met with limited success [39], which indicates a need to explore other less invasive options for screening. One proposed option is serology testing for overexpression of HPV E6/E7 oncoproteins. The overexpression of E6/E7 oncoproteins corresponds with serological data, which, in conjunction with other suspected infections such as HIV may allow clinicians to identify women who are at high risk of cancerous lesions of the cervix earlier than currently identified.

In addition, a serological test for screening may be more acceptable to patients and technically easier to conduct for health care professionals than a Pap or related smear. So far, in South Africa, no large serological studies have been done on the overall relationship between HPV-16 and -18 (L1, E6 and E7) antibodies and cervical cancer.

1.4. Significance of the study

This study is of public health interest in South Africa. Most older teenagers and adult women who missed the opportunity for HPV vaccination are still at risk for developing cervical cancer in the next 30-40 years. With the advent of rapid antibody testing, this study has the potential to inform research into the optimisation and development of less invasive screening methods using, for example, a (yet to be developed) finger prick device or using routinely collected blood at antenatal clinics [40,41]. In this way, it could influence the introduction of HPV-related cancer-screening tools and the refinement of screening guidelines. Based on the findings of this study, This PhD will make recommendations to lobby for policy change and to champion new research into HPV-serology screening programmes. If successfully translated into an effective detection tool, this effort could ultimately result in the downstaging of cervical cancer disease at diagnosis. Additionally, the findings from this PhD would;

1. Increase the understanding of the relationship between HPV-antibodies and cervical cancer.
2. Add to the limited knowledge of the distribution of HPV types amongst cervical cancer cases in South Africa through serological biomarkers.
3. Provide avenues for new research on the feasibility of implementing HPV serological biomarkers in a high-risk population.

1.5. Literature Review

1.5.1. The HPV Genome and Carcinogenesis

The HPV comprises a double-stranded circular DNA genome ~8kb in length. The DNA strand codes for eight genes and a noncoding region (URR) that regulate viral replication and controls cellular and viral transcription. The genes are divided into early (E) and late (L). The E genes are E1, E2, E3, E4, E5 and E6. The L genes comprise L1 and L2. The L genes encode the major (L1) and minor (L2) capsid proteins (Figure1- 5).

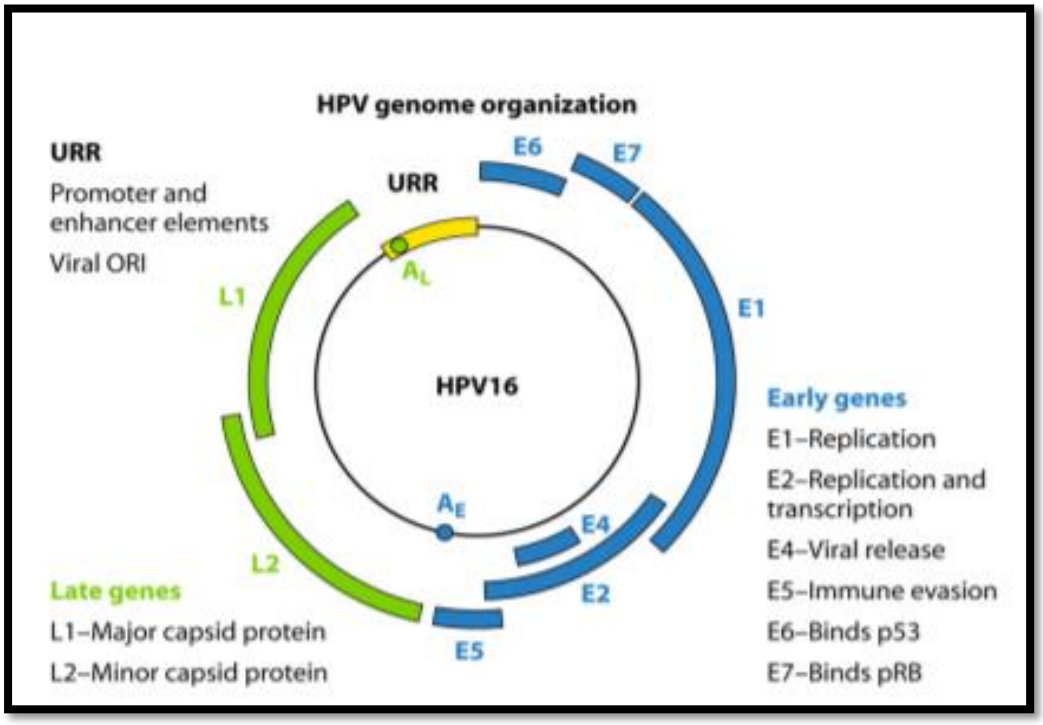


Figure 1-5:Structure of the HPV GENOME. The URR is the upstream regulatory region. The Early genes have E1-E7. The late genes have L1-L2. Reproduced with permission from the journal Source Stanley [42]

Figure1-6 shows the initial steps for HPV L1 and L2 during HPV infection. HPV L1 is a viral nuclear protein that encloses the HPV-DNA to create new infectious particles [43]. However, after a natural HPV infection, the minor capsid protein L2 does not elicit a humoral response to antibodies against HPV L2 protein, as the L2 is transient on the capsid [44,45].

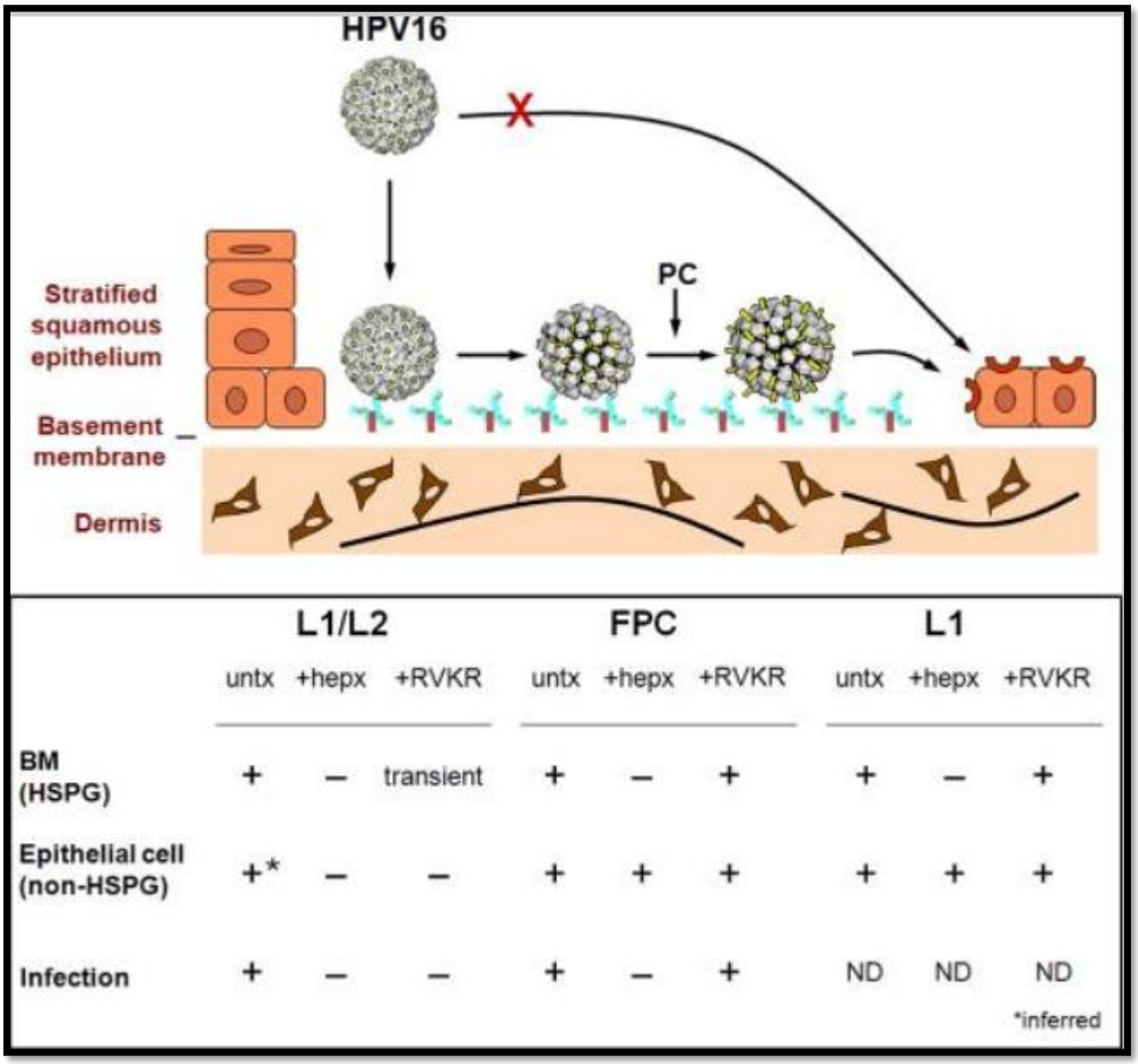


Figure 1-6: Initial steps for HPV infection and L1 humoral response: A model for occurrence resulting in vivo infection. Untreated, mature L1/L2 binds to heparan sulfate proteoglycans (HSPG) on the basement membrane (BM). This interplay results in a conformity change that permits the availability of the L2 N terminus to furin proprotein convertase (FPC). The bottom part of the figure shows the binding patterns of the capsids. Untx =untreated tissue, hepx=heparise treated tissue, RVKR=proprtein convertase inhibitor decanolyl-RV, and ND=the experiment was not done. Reproduced with permission from the Journal: Source*: Kines*: Source: Kines [44].

The E5, E6 and E7 oncoproteins are essential for viral proliferation as well as the formation of tumours [46]. Following exposure to hrHPV, the viral genome may integrate into the genome of differentiating epithelial cells, resulting in early protein expression [47]. The E5 oncoprotein plays an important role in

the early phases of carcinogenesis. It enhances the possible immortalisation of E6 and E7 proteins. The overexpression of E6 and E7 oncoproteins from hrHPV is associated with disrupting the cell cycle process and contributes to the transformation of malignant cells [48]. In particular, the presence of E7 in human cells prevents phosphorylation of the retinoblastoma (Rb) (a key cell regulator) protein leading to uncontrolled cell production [26].

Similarly, the E6 protein binds to the E3 ubiquitin ligase E6-associated to E6AP by sending it to the ubiquitin-dependent proteolytic pathway, provoking the p53 proteins (proteins involved in the control of a cell cycle process, including apoptosis), causing antiapoptotic state [27]. Figure 1-7 below illustrate the malignant cell transformation process. These oncoproteins result in the immortalisation of cervical cancer cells [49]. They also cause cell proliferation, which elevates anti-HPV E6 and E7 antibodies in serum [49]. Figure 1-8 is a framework of events associated with mucosal hrHPV infection in the genital tract.

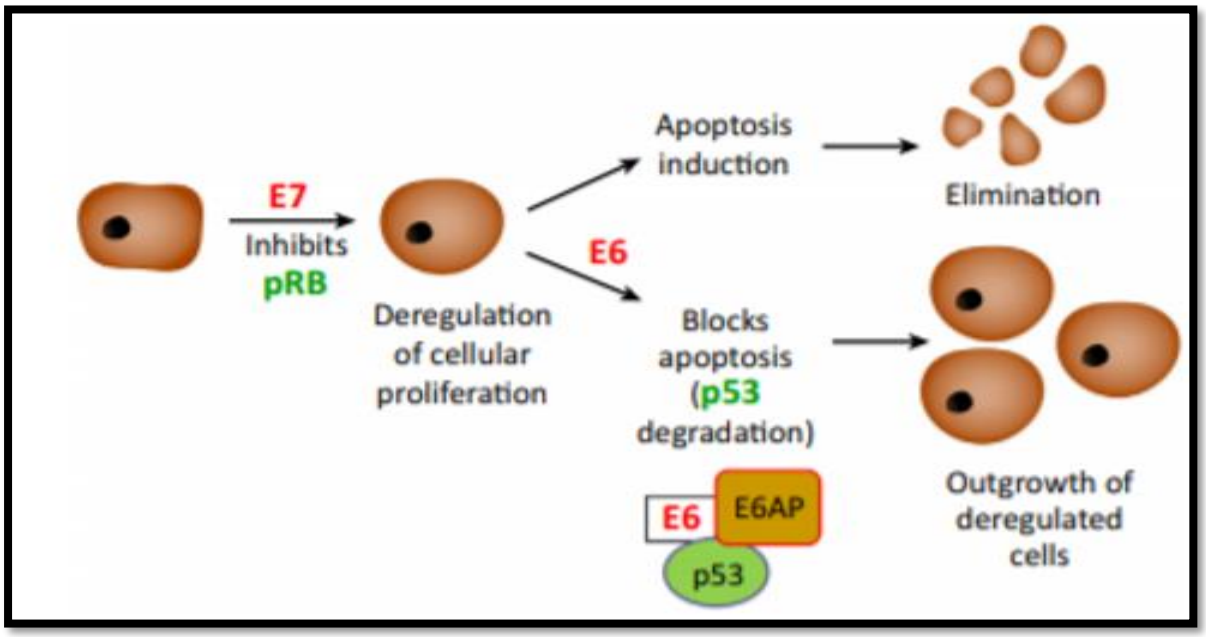


Figure 1-7: HPV E6 and E7 oncoprotein during malignant cell transformation: Expression of E7 in human cells prevents phosphorylation of the retinoblastoma (Rb) (a key cell regulator) protein leading to uncontrolled cell production. The E6 protein binds to the E3 ubiquitin ligase E6-associated to E6AP by sending it to the ubiquitin-dependent proteolytic pathway, provoking the p53 proteins (proteins involved in the control of a cell cycle process, including apoptosis), causing antiapoptotic state (an outgrowth of deregulated cells), reproduced with permission from the journal Source: Hoppe-Seyler. Source [50].

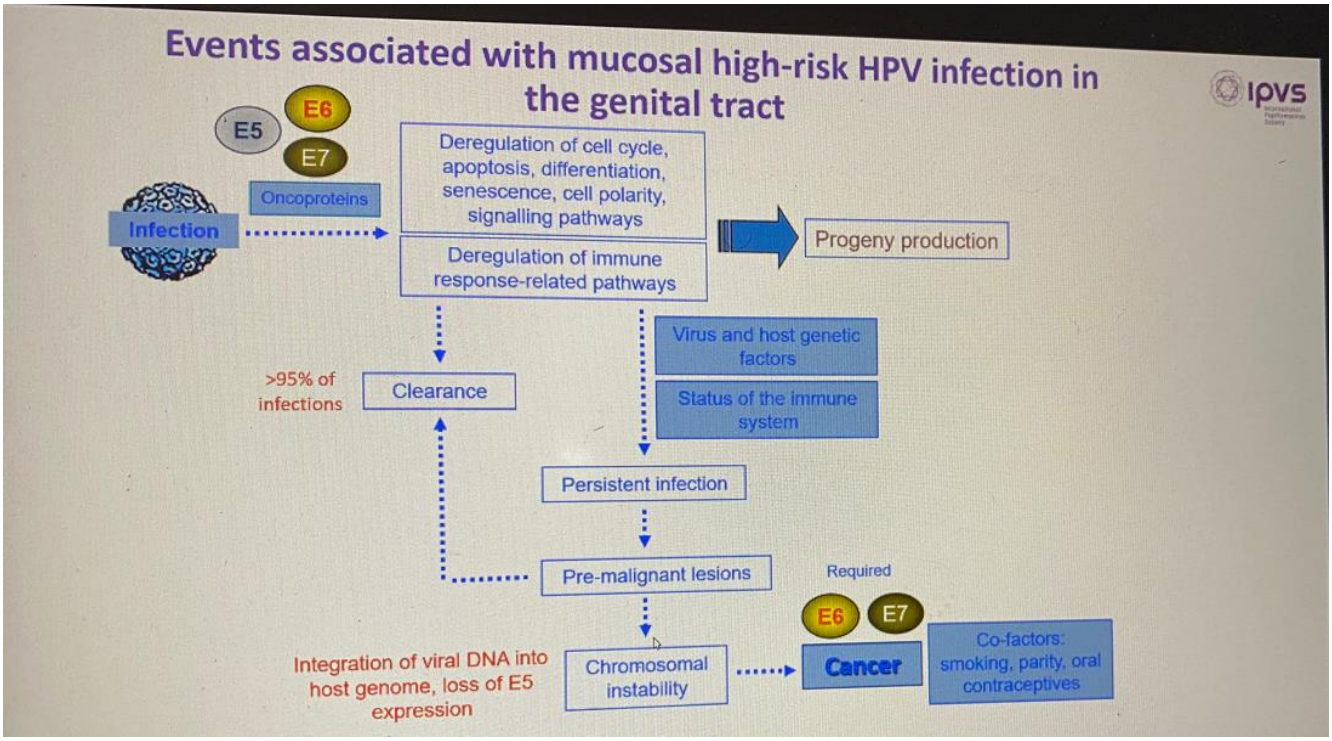


Figure 1-8: The pictograph for a framework of events associated with mucosal hrHPV infection in the genital tract. This picture was taken during a Webinar on the topic "What does it take to decide if a cancer is caused by HPV?" (21 September 2022). Dr Massino Tommasino presented it. The picture was taken on 21 September 2022 (M.G. Singini) and used with permission.

1.5.2. HPV detection.

Since infection with HPV can take a long period in an asymptomatic state, it is difficult for an infected person to realise that they have a virus until the clinical presentation of the disease [42]. To date, advancement in knowledge on HPV has facilitated the detection of HPV at the earliest stage. Different detection methods have been developed, direct (the tests are at a molecular level) and indirect (visual inspection and cytology).

The direct methods (HPV DNA) include; Southern blot, in situ hybridization, hybrid capture II, dot plot and a standard polymerase chain reaction (PCR) [51]. As an endpoint, PCR is not used to measure viral

load. This is done by qPCR methods that measure real-time the amplification from which the initial template amount can be estimated. HybridCapture assay generally detects HPV in cervical smear samples and not in tissue samples. It is, at most, semi-quantitative. Both latent AND persistent infections can be detected by such methods, as they amplify (PCR) or detect byprobes (HybridCapture) HPV DNA. However, these different states of latency versus persistency cannot be discriminated [52]. There are also variations in the sensitivity and specificity of HPV DNA varies. The variation is due to the type of samples collected, storage, DNA extraction and the range of HPV types amplified [53]. Other direct methods (protein) include Nucleic acid amplification (NAAT) and HPV Ribonucleic acid (RNA). The HPV E6/E7 messenger RNA (mRNA) is a commercially available test, but it detects the RNA encoding for the oncoproteins [54].

The indirect methods for detecting HPV infection involve assessing the outcome of the disease on mucosal cells. The indirect methods include; Pap test, Liquid-based cytology (LBC) and Visual inspection (VIA). Dr George Papanicolaou first developed in 1928. In many developed countries, it was considered a gold standard for detecting abnormal cells in the uterine cervix. Samples are taken from the uterine cervix using a speculum/brush and put on a glass slide for examination using a microscope. The aim is to detect abnormal cells and remove them at the earliest stage [55].

VIA

The method involves directly inserting a vaginal speculum and applying acetic acid or Lugol's iodine (VILI) to the uterine cervix. After that, a cervix examination is done with the naked eye to assess if there is a colour change [56]. Studies have shown that VIA is moderate sensitivity, non-invasive, easy to use and cheap for low -and- middle-income countries where Pap test programs remain a challenge [57,58].

However, the limitation of VIA is low specificity [56]. To overcome such limitations, multiplex serological testing for HPV oncoproteins antibodies offers promising solutions for detecting HPV-related cancers [37]. A multiplex HPV antibodies test has previously shown high specificity and is inexpensive to run in limited resource settings [59]. Other HPV antibody tests include HPV serology and ELISA. The WHO considers HPV serology a future screening test (Table 1- 1) [60]. Table 1-2 presents some of the HPV antibody test's advantages and disadvantages.

Table 1-1: Three approaches to cervical cancer screening and future tests. Source: WHO [60].

Molecular	Cytologic	Visual inspection
Nucleic acid amplification tests (NAAT)[a] » high-risk HPV DNA/ NAAT » mRNA **DNA methylation[b]** **Protein biomarkers[b]** » HPV antibodies » oncoproteins	**Conventional Pap smear[a]** **Liquid-based cytology (LBC)[a]** **Dual staining to identify p16 and Ki-67[a]**	**Visual inspection with acetic acid or with Lugol's iodine (VIA/VILI)[a]** » naked eye » magnified by colposcope or camera **Automated visual evaluation of digital images[b]**

[a] Current tests
[b] Tests under evaluation (future tests).

Table 1-2: Different HPV antibody tests Advantages and Disadvantages

Assay name	Advantage	Disadvantage	references
HPV-serology (Virus Like Particles from L1)	- Critical for seroepidemiological studies and monitoring of HPV vaccines. - Measures antibodies' response to specific HPV types. - All immunoglobulin classes are detected	- Unable to Multiplex. - Time-consuming - Involves much labour	[61]
ELISA	- It is quick and has a high throughput - Low antigen - Easy to maintain - Amenable to multiplex serology assay	- Can only detect one immunoglobulin class at a time, measured by antibodies - Can detect non-neutralising antibodies	[62]
Multiplex HPV Serology	- Based on recombinant glutathione S-transferase (GST) fusion proteins. - Detects antibodies to multiple HPV types at the same time. - Fast and high throughput - Measures all classes of immunoglobulin	- Neutralizing monoclonal antibodies of a specific type are required - Detection of neutralising antibodies is in a small amount	[37,62]

1.5.3. Attributable fraction of HPV types in cervical cancer.

Almost all invasive cervical cancer is attributable to HPV infection globally [63]. HPV-16 and -18 contribute to at least 70% of invasive cervical cancer [64]. Serraino et al [65] reported the attributable fraction for each of the common hrHPV genotypes; HPV-16 (60.6%), HPV-18 (10.2%) and HPV 45 (5.9%). The three hrHPV genotypes are more likely to integrate into the human genome [66]. In addition, most cervical cancers are caused by HPV-16 or -18, with hrHPV types 31,33,45,52 and 58 playing a lesser role [67]. Figures 1-9 and 1-10 show maps of cervical cancer attributable to HPV infection globally and in Africa.

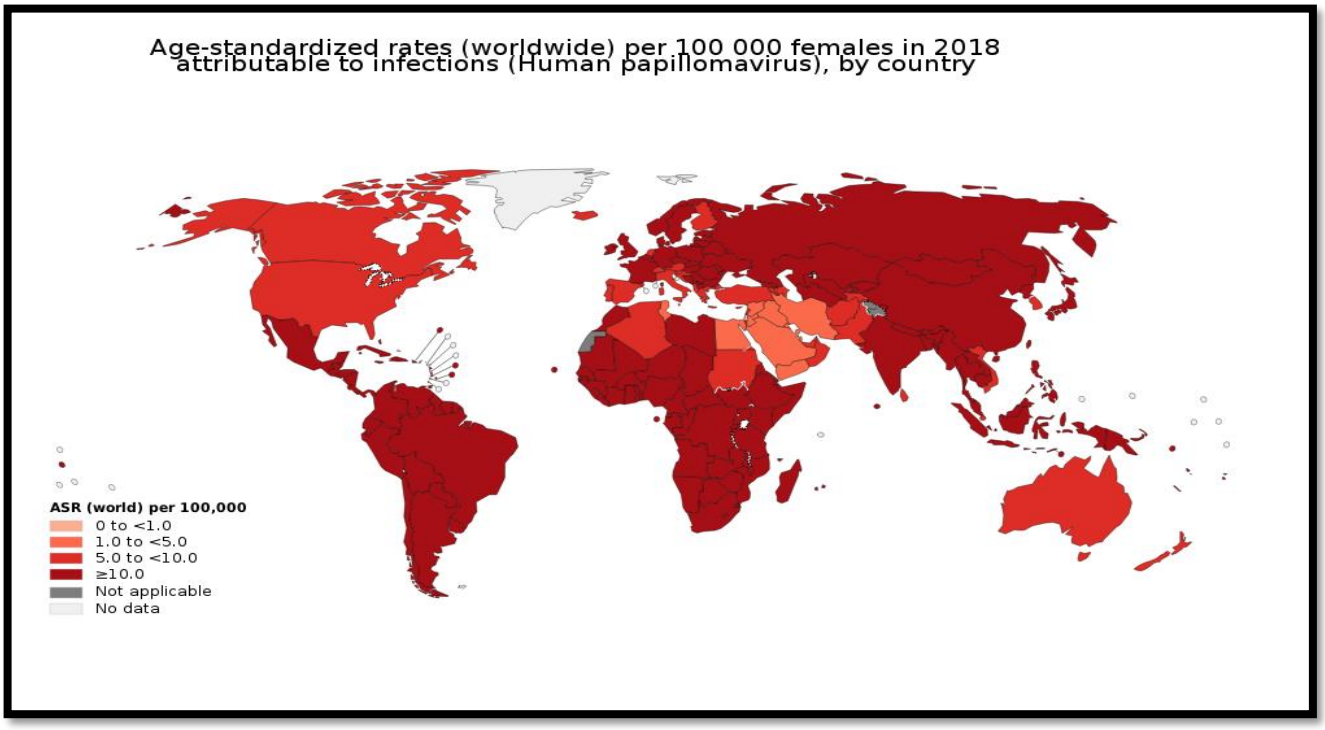


Figure 1-9: Estimated age-standardized rate for cancers attributable to HPV among women globally. Reproduced with permission from IARC: Source GLOBOCAN [2].

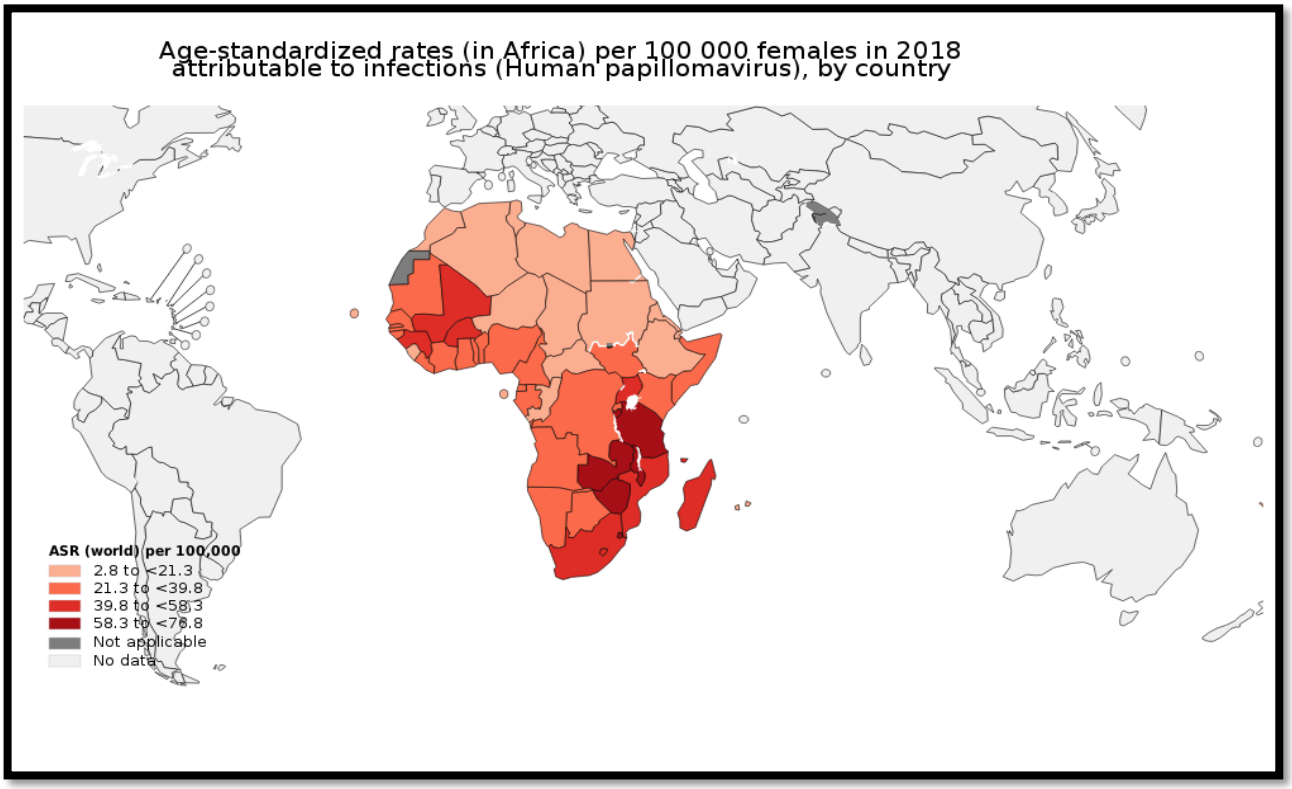


Figure 1-10: Estimated age-standardized rate for cancers attributable to HPV among African women. Reproduced with permission from IARC Source GLOBOCAN [2].

1.5.4. Approaches to secondary prevention of cervical cancer.

1.5.4.1. Cancer screening

Screening for cancer is an effective secondary prevention strategy to prevent cancer occurrence. It involves early identification of premalignant lesions before clinical manifestation through non-invasive, cheap and user-friendly screening tests, ultimately leading to improved health outcomes [68]. A screening test differs from a diagnostic test in that a screening test is applied to asymptomatic individuals, usually to identify individuals at risk of the disease in the general population [69]. In contrast, the diagnostic test is for diagnosing symptomatic individuals needing health –care services to detect the disease [70,71].

1.5.4.2. Principles of disease screening

In 1968, Wilson and Jungner [69] identified ten principles and practices to consider in screening for a disease. The WHO adopted the guidelines, which are still valid today. Table 1-3, on page 21, provides a list of principles and practices in screening for disease. They are grouped into four areas; (a) the disease, (b) the screening test, (c) treatment for the condition and (d) the screening program [72].

Table 1-3:List of Principles and Practices in Screening for disease

Box 1. World Health Organization Screening Guidelines
A. The disease
i. The disease should be an important health problem.
ii. The natural history of the disease, including development from latent to declared disease, should be adequately understood
iii. There should be an identifiable latent and early stage.
B. Screening Test
iv. The test should be suitable for screening.
v. The test should be acceptable to the population.
C. Treatment for the condition
vi. There should be an accepted treatment for patients with known condition.
D. Screening program
vii. There should be an agreed policy on whom to treat as patients.
viii. There should be available facilities for diagnosis and treatment of the condition resulting from screening.
ix. There should be a cost-benefit analysis on case-finding (including diagnosis and treatment of patients diagnosed) in relation expenditure on medical care as a whole.
x. Passive and Active case detection should be a continuing process and not a "once and for all" project.

Source: Wilson and Jungner [69]

1.5.4.3. Cervical Cancer Screening

Screening asymptomatic women contribute to detecting early cervical cancer and pre-malignant lesions, reducing the incidence and mortality rates[73]. Since 1960, cervical cancer incidence and mortality rates have decreased by at least 80% in HICs, due to organized cervical cytology-based screening programmes [74]. However, the cytology test remains controversial due to its low sensitivity but higher specificity. The WHO recommended cytology (Pap test) screening interval guidelines to repeat the cytology every 3-5 years among women aged 30-65 years [75].

In the last decade, there has been a consideration from different countries (including South Africa) to replace the cytology test with HPV tests as a primary test. HPV tests have shown greater sensitivity and negative predictive value than conventional cervical cytology. Conventional Pap smears have a sensitivity of 54% and specificity of 97% in detecting cervical atypical squamous cells of undetermined significance (ASCUS) or worse [76]. HPV DNA testing of cervical tissue is gaining popularity, but coverage in LMIC settings remains low [77]. Findings from systematic review and meta-analysis showed that using HPV-self-sampling tests among women aged 30-64 years was associated with increased cervical cancer screening uptake in HICs [78]. However, uptake was still low in LMIC.

1.5.4.4. Cervical Cancer Screening Programmes in South Africa

Several attempts have been made in South Africa to implement cervical cancer screening programmes at the national, provincial and grassroots levels [12]. In the 1970s, there was a suggestion by the Department of Health that Pap smears should on be done on women only if the cervix looked abnormal [79].

In the 1980s, the different cervical cancer programmes introduced in urban areas failed due to low turn-out among women at the health facility, lack of education and delayed programme implementation [12,20]. However, in 2000, a national cervical cancer screening policy was introduced by the Department of Health [20].

Although the South African government launched the national cancer screening policy, the implementation is partial. At present, liquid-based cytology is the primary screening method for South Africa, as recommended by the Department of Health's new national cervical cancer screening policy of 2017 [17]. The implementation of liquid-based cytology is currently in seven provinces, except Limpopo and Western Cape, which still use conventional cytology. In addition, the new policy recommends that all asymptomatic women aged 30 years and above who are HIV- negative or do not know their HIV status should be screened for cervical cancer every ten years until the age of 50 years. Another screening programme available in South Africa is the diagnostic programme, where all women with gynae complaints are screened for cervical cancer. In 2016, the South African government adopted the Universal Test and Treat programme, where every woman who tests positive for HIV gets screened immediately, regardless of age [17].

1.5.5. The utility of HPV-16 and -18 (E6 and E7) antibodies in cervical cancer screening.

Current methods for screening HPV-related cervical cancer using cervical smears or direct visualization of the cervix have had limited success in LMIC settings [80]. Besides, deficiencies in the availability of cytology services and subsequent follow-up and treatment, poor knowledge dissemination and personal and cultural factors have contributed to the failure to replicate the benefits observed in HIC. So far, blood-based antibody tests of hrHPV (especially 16 and -18) and early oncoproteins (especially E6/E7)

have shown potential in the detection of cervical cancers [37]. A yet-to-be-developed less-invasive HPV-16 or -18 E6 and E7 antibody screening tool is a proposed test that uses serological markers. The test could be administered during routine visits. It may improve cervical cancer screening rates, thereby increasing the detection of the disease in at-risk populations. The HPV-16/18 E6 and E7 test provides a solution to a point-of-care screening, which may be an alternative test for use in LMIC settings where cytology screening program coverage is still poor. Figure 1-11 shows the patient flow for the HPV-serology point–of–care.

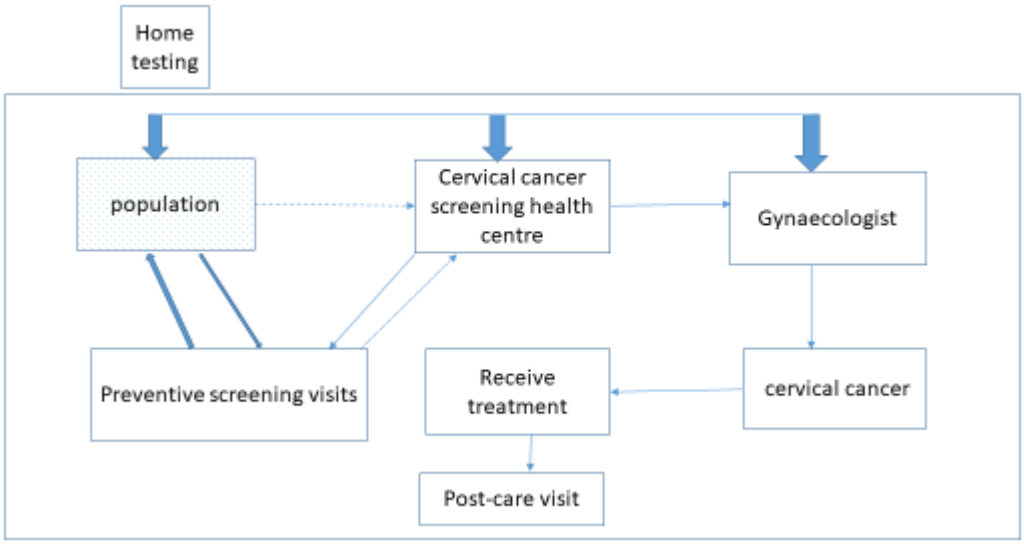


Figure 1-11: illustration of patient flow for point-of-care test

Also, the HPV-16/18 E6 and E7 antibody assay may not require a complex procedure for screening for cervical cancer. By using blood drawn from women attending a point of care, obtaining results may be within the same day. Figures 1-12 and 1-13 illustrate how HPV serology tests could reduce patients waiting time compared to HPV-DNA/ Cytology screening. Figure 1-14 shows how the HPV E6 and E7 antibody tests could be used to screen for cervical cancer.

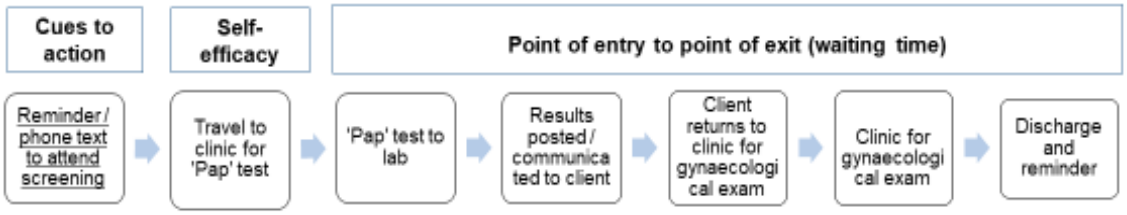


Figure 1-12: HPV DNA/ Cytology screen patient flow

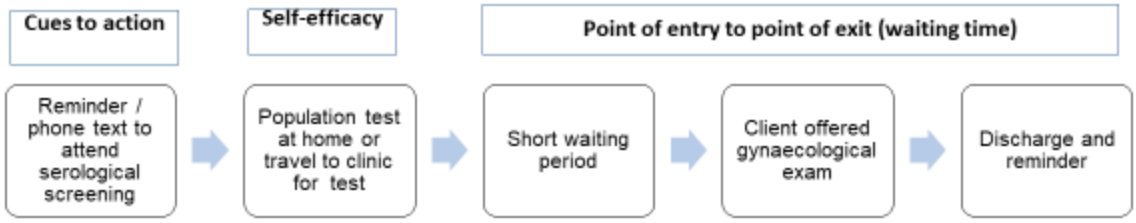


Figure 1-13: HPV serology screening patient flow

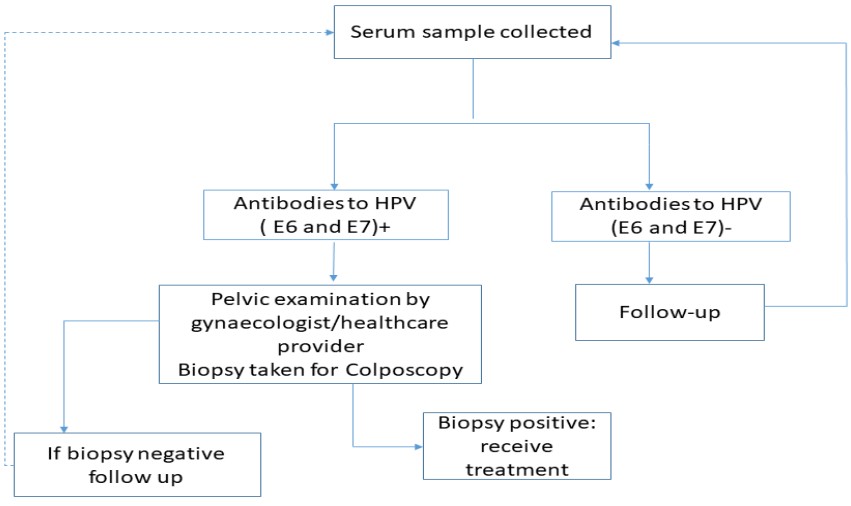


Figure 1-14: A proposed HPV E6 and E7 antibody test for cervical cancer screening. The Figure does not include the follow-up time interval because the test's negative predictive value had not been validated.

1.5.6. The seroprevalence of antibodies to HPV-16 and -18 (L1 and E6 and E7) proteins by socio-demographic and sexual history and their association with HIV.

Previous case-control studies have shown antibodies to HPV-16 and -18 (L1 and E6/E7) more prevalent in cervical cancer cases than controls [81,82]. Research has shown a seroprevalence range of 21% to 53% for HPV(L1 and E6/E7) antibodies among HIV-negative cervical cancer patients [83,84]. A variation in seroprevalence of anti-HPV antibody levels may depend on different exposures, socio-demographic characteristics in diverse populations, and the test used [30]. Previous studies have shown an upward age trend among women with HPV-16 and -18 seroprevalences using ELISA assays [85–88]. In China, among women aged 35-44 years without cancer, a seroprevalence of HPV-16 (6.9%) was reported [85]. Similarly, in Germany, a study that used a multiplex HPV serology assay (GST) found a seroprevalence of HPV-16 (9.6%) and -18 (6.9%) antibodies among women aged 30-39 years [89]. In a study from the United States of America (USA), the seroprevalence of HPV-16 and -18 peaked at 25% among women aged 30-39 years [86–88]. The possible explanation for an increase in HPV-16 and -18 antibody seroprevalence with an increase in age could be the number of sexual partners [87,88,90]. However, most of the studies on antibodies to HPV (E6 and E7 and L1) seroprevalence have primarily been from developed countries and other populations [59,91–93], where the burden of HIV is low. In South Africa, little is known about HPV E6 and E7 antibodies in controls and their association with HIV.

1.5.7. HPV-16 and -18 L1 E6 and E7 proteins antibody seropositivity and cervical cancer risk in HIV-Positive and HIV-Negative Black South African women.

Previously published studies from other populations of different geographical settings have shown different results regarding HPV antibodies and cervical cancer [21]. In Africa, only two studies (Tunisia

and Uganda) have been published on the association between HPV antibodies and cervical cancer[84,94]. In Uganda, (N=884) anti-HPV-16 antibodies were associated with cervical cancer [94]. However, it only focused on the IgGL1. The Tunisian study (N=134) did not assess the association between HPV E6/E7 oncoproteins and cervical cancer [84]. The major limitation of the two mentioned studies is that they both used a different ELISA technology for detecting HPV antibodies. Small sample sizes limited both studies, and they were conducted at other laboratories with different cut-off points. Hence, making a comparison of results difficult.

In South Africa, local data are still lacking on the importance of HPV (L1, E6 and E7) proteins and cervical cancer. A previous study that reported on HPV (L1, E6 and E7) serological markers using a multiplex serology approach investigated the role of HPV on oesophageal cancer showing null results [95]. This PhD aimed to provide further evidence on the part of HPV-16 and -18 late-early proteins on cervical cancer in the Black population in South Africa using readily available data from the JCS.

1.5.8. Lifestyle risk factors associated with cervical cancer

The main route for transmission of HPV is through sexual intercourse [96]. In about 90% of infected individuals with HPV, the infections do not persist and clear off by the immune system within two years after exposure [48]. About 10% of patients with persistent HPV infections of the cervix develop premalignant lesions [48,97]. However, less than 1% of these pre-cancerous lesions develop into invasive cervical cancer [98]. A combination of viral and lifestyle factors increases the risk of HPV-driven malignant progression to cervical cancer [99]. Sexually transmitted infectious agents such as HIV increase the risk of HPV persistence [100]. For instance, HIV significantly increases the risk of developing

cervical cancer by 1.6 [38]. Lifestyle factors include hormonal contraceptives, smoking, alcohol and parity, which contribute to HPV persistence in the cervix [101].

1.5.9. Conceptual Framework

The conceptual framework guiding this study in Figure 1-15 below was informed through a synthesis of the literature on the relationship between HPV (L1, E6 and E7) antibody levels and Cervical cancer among the adult population. It is a modification of the conceptual framework on the causes of oral cancer [102]. As shown in Figure 1-15, Warnakulasuriya [102] grouped causes of oral cancer into modifiable, non-modifiable and emerging risk factors. However, Warnakulasuriya [102] did not explore whether or not HPV oncoproteins antibody could play a mediation role in the development of cervical cancer. This PhD went a step further by focusing on HPV (L1, E6 and E7) and HIV antibody responses and cervical cancer in adult South African Black women.

The aetiology of cervical cancer is multifactorial, with several established exposures (i.e. HPV) known to be necessary for their causation. Individuals with compromised immune systems, such as HIV-infected, are at higher risk of developing cervical cancer [103]. HIV may re-activate a latent oncogenic HPV that may lead to cervical cancer progression [81]. Previous studies have shown that the number of sexual partners influences the acquisition and persistence of HPV [31,96]. However, other studies have shown an association between contraceptive use (i.e. consistent condom use) and higher education levels with a lower risk of HIV infections among women [104,105]. Studies on behaviour have shown that tobacco smoking and alcohol consumption are associated with many lifetime sexual partners [106]. In addition, Eldridge [107] found an association between smoking and increased levels of HPV infection. In a pooled analysis of HPV-16 E6 antibodies, Kuhs et al. [108] found that being a former smoker was

strongly associated with HPV-16 E6 antibodies (OR=5.5; 95% CI:1.2-51.8). Cervical cancers are prevalent in women aged 34-54 years [36,109]. However, premalignant lesions commonly develop among women living with HIV at a younger age. They may progress to invasive cervical cancer within a limited space [110].

Studies worldwide, including those from the JCS in South Africa, found an association between smoking and cervical cancer [111–113]. Tobacco smoking suppresses the immune system of the host against HPV [114]. Other studies argue that in tobacco smoke, there are polycyclic aromatic hydrocarbons that are carcinogenic and could contribute to malignant transformation [115]. Concerning alcohol consumption, a Swedish study found an association between alcohol consumption and cervical and vaginal cancers [116]. In Africa, some studies have reported an association between local beer consumption and cervical cancer [117]. In Lesotho, Martin and Hill [118] found an association between alcohol consumption and cervical cancer (local beer (frequent drinking vs never RR=3.2) and European beer (frequent drinking vs never RR=1.9). In Bulawayo, Zimbabwe, Parkin et al. reported an increase in cervical cancer (Adjusted Odds Ratios (AOR)=1 .6, 95% CI: 1 .3-1 .9) among women who were frequent alcohol drinkers [119].

1.6. Research Question and Objectives

1.6.1. Research question

Are serological markers comprising HPV early and late proteins useful in detecting cervical cancer?

1.6.2. Overall Aim

The main aim of this study was to assess the importance of the HPV16 and -18 late and early oncoproteins and cervical cancer risk factors among adult Black South African women, using data from the JCS.

1.6.3. Specific objectives:

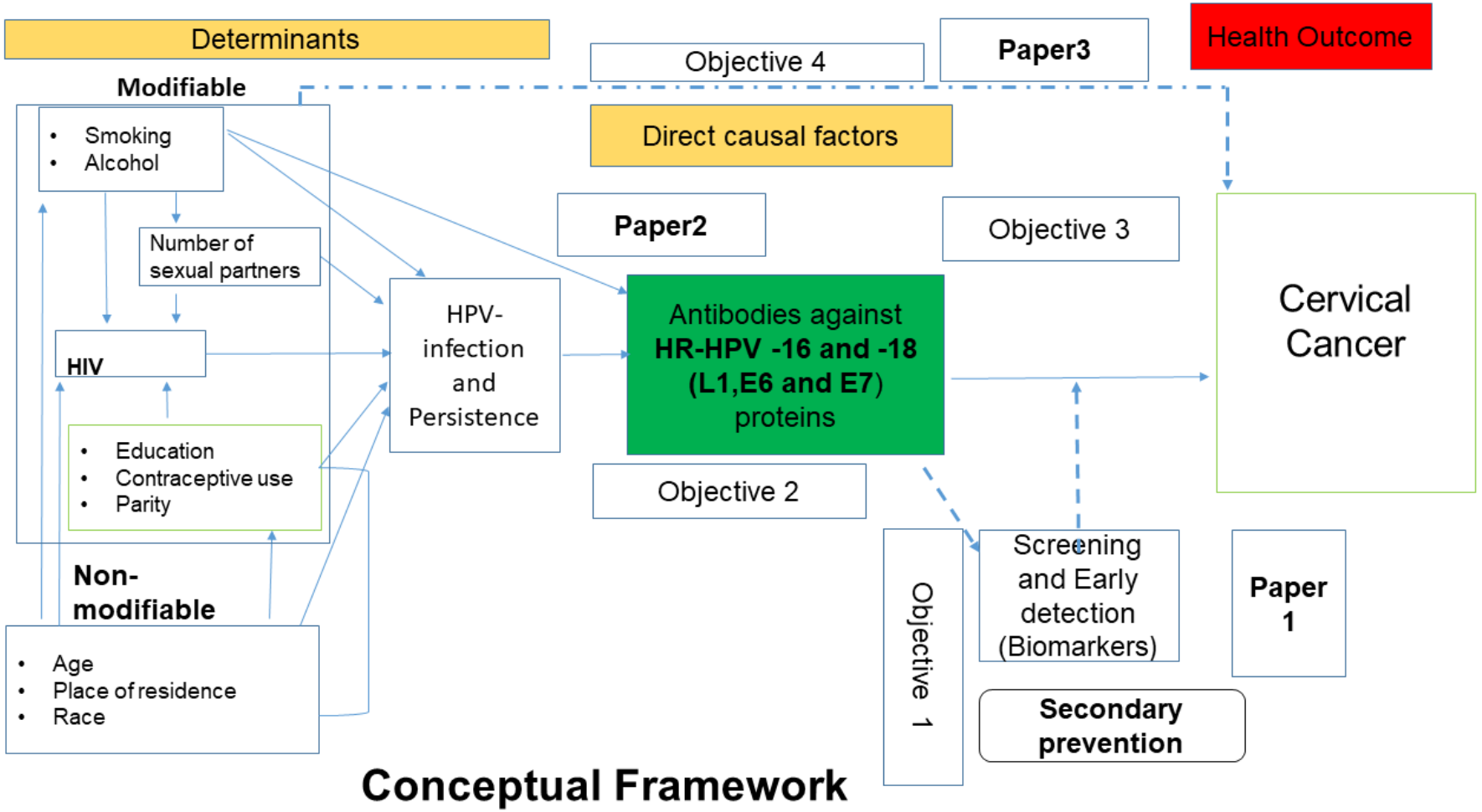
Using a well-described case-control design, the specific objectives were:

I. To investigate the utility of antibody testing for HPV-16 and -18 (E6 and E7) in cervical cancer screening (Chapter 3).

II. To calculate the seroprevalence of antibodies to HPV-16 and -18 (L1 and E6 and E7) proteins among cancer controls by socio-demographics and sexual history and assess their association with HIV (Chapter 4).

III. To assess the association between HPV-16 and -18 (E6 and E7 and L1) antibodies and cervical cancer in HIV-positive and HIV-negative patients (Chapter 4).

IV. To evaluate the contribution of several known lifestyle risk factors for cervical cancer among Black South African women (Chapter 5).



Source: Adapted from Warnakulasuriya [102].

Figure 1-15: A conceptual framework for studying hrHPV (L1, E6 and E7) antibodies and Cervical cancer.

2.0. Chapter Two: Methodology

This chapter provides the study setting and design, study population, definition of cases and controls, statistical analysis plan, ethical consideration and funding. This study used data from the JCS.

2.1. Study design and setting

We used data from the JCS to conduct an unmatched case-control study analysis among adult Black South African cancer patients. In this analysis, cases were participants with cancers of the cervix (aged 25-64), and controls were participants (aged 25-64 years) with cancers unrelated to the exposure under study (i.e. infections) [33]. Given that the data were already collected, matching was no cost savings. Hence, JCS lends itself to an unmatched case-control study design (with age adjustment at the analysis stage). We compared the serological profiles of cervical cancer patients with the serological profiles of infection-unrelated cancer (control) patients. Below is the map for Johannesburg and surrounding areas.

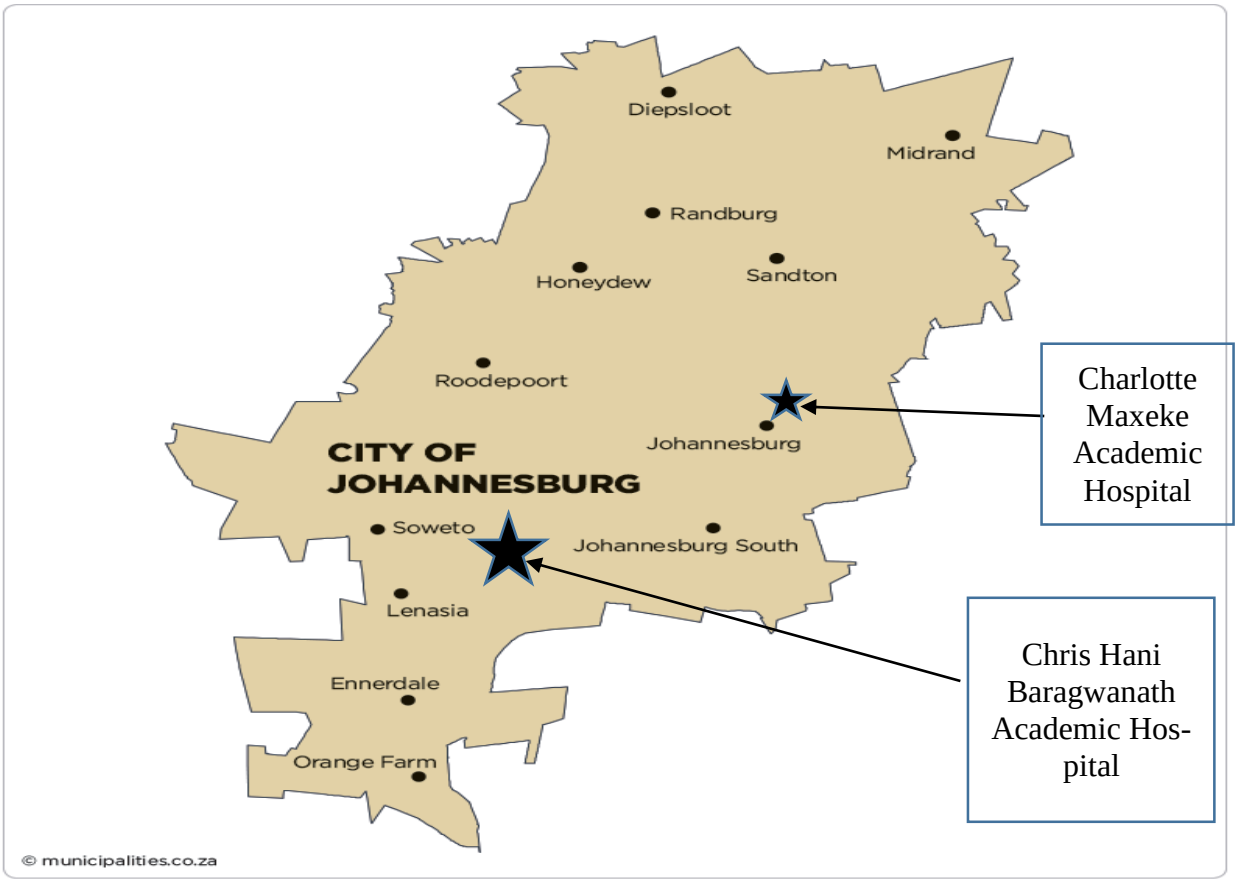


Figure 2-1:Map of Johannesburg (Adapted from https://municipalities.co.za/map/2/city-of-johannesburg-metropolitan-municipality)

2.2. Study population

Participants for this study were Black South African cancer patients who were newly diagnosed with any cancer at the primary oncology and radiation therapy clinics situated in the largest provincial tertiary hospital (Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg, and associated satellite hospitals, mainly Chris Hani Baragwanath Academic Hospital in Soweto). A detailed description of the JCS has been published elsewhere [33]. Briefly, about 26,263 (8,677 males and 17,586 females) participants with newly diagnosed cancer consented to participate in the JC study from 1995-2015. Participants

were recruited, provided written or witnessed verbal consent, interviewed using a structured questionnaire and provided an optional blood sample before receiving chemo – or radiation therapy. Because histological confirmation is a requirement before cancer treatment at the medical oncology and radiation therapy departments (where recruitment took place), over 95% of participants had their cancer type verified by histopathology [33].

2.3. Definition of cases and controls

The JCS is amenable to a case-control design, using controls unrelated to the exposures of interest [33]. The cases and controls were coded using the International Classification of the Diseases for Oncology (ICD-O-3/ICD-10): The cases for this data analysis were women who were newly diagnosed with cervical cancer (C53) (n=3,540).

Controls (n=5,709) were female patients newly diagnosed with cancers with no known relationship with any infectious agents. These were Breast (C50) (n=3,843), Colorectal (C18-20) (n=340), Oesophageal (C15) (n=314), Ovarian (C56) (n=263), Endometrial (C54-55) (n=224), Lung (C33-34) (n=159), and Pancreatic cancer (C25) (n=51), Myeloid Leukaemia (C92) (n=105), Myeloma (C90) (n=123), and Other minor cancer types (each comprising $n < 50$).

2.4. Statistical Analysis

A detailed description of statistical approaches is in each paper of the PhD thesis chapters. We used the following method: a bivariate hierarchical random effects model approach for the meta-analysis in chapter three, unconditional logistic regression models in chapters four and five and Miettinen's Population Attributable Fraction methods [120] in chapter five.

2.5. Ethical considerations

The JCS and the current study were approved by the University of the Witwatersrand Human Research Ethics Committee (HREC). (Medical) (certificate number for the present study is M200252). In the JCS, participants gave written informed or witnessed consent to a once-off interview and optional blood draw and anonymised their information and blood sample. Any future investigations require approval of the University of the Witwatersrand HREC.

2.6. Funding

The funders were not involved in the manuscript's conceptualization, review or approval. This project forms part of an international research program to identify evolving risk factors for cancer in African populations (ERICA-SA). (http://www.mrc.ac.za/intramuralresearch-units/evolving-risk-factors-cancers-africanpopulations-erica-sa)

PART 2

EMPIRICAL CHAPTERS

3.0. Chapter Three: Usefulness of high-risk HPV early oncoprotein (E6 and E7) serological markers in the detection of cervical cancer: A systematic review and meta-analysis

Authors: Mwiza Gideon Singini, Elvira Singh, Debbie Bradshaw, Wenlong Carl Chen, Melitah Motlhale, Abram Bunya Kamiza, Chantal Babb de Villiers, Mazvita Muchengeti, Christopher G. Mathew, Robert Newton, Noemi Bender, Tim Waterboer and Freddy Sitas.

Published in Journal of Medical Virology (J Med Virol. 2022;1-10).

3.1. Introduction

In this chapter, we conducted a systematic review and a meta-analysis to assess the utility of HPV-16 and -18 E6 and E7 oncoproteins serological markers for detecting cervical cancer. The chapter discusses how current screening methods for cervical cancer in LMICs have been met with limited success among women. It further identifies novel screening methods for cervical cancer in limited settings. The chapter has four parts; the background, methods, results and discussion sections. The systematic review and meta-analysis was published in the Journal of Medical Virology (J Med Virol 1-10)

The main contribution of chapter three to the overall aim of the PhD thesis was the investigation of the usefulness of antibody testing for HPV-16 and -18 (E6 and E7) in cervical cancer screening. It was essential to start with a systematic review and meta-analysis to answer this critical question. This helped identify the deficiency in the literature on the HPV-16/18 oncoproteins serological markers and estimate their sensitivities and specificities.

Abstract

Background: We reviewed the literature on the importance of selected anti-hrHPV-antibodies (namely16/18 and early oncoproteins, E6 and E7) as potential serological markers for the early detection of individuals at high risk of cervical cancer.

Methods: We searched for studies in PubMed and Embase databases published from 2010 to 2020 on antibodies against hrHPV E6 and E7 early proteins and cervical cancer. Pooled sensitivity and specificity for HPV-16 and -18 antibodies were calculated using a bivariate hierarchical random effect model.

Results: A total of 69 articles were identified; we included 3 studies with 1,550 participants. For the three HPV-16/18 E6 and E7 antibody tests, ELISA-based assays had a sensitivity of detecting CIN2+ of 18 % (95% CI:15-21) and specificity of 96% (95% CI:92-98), and for Slot Blot sensitivity was 28.9% (95% CI: 23.3-35.1) and specificity 72% (95% CI:66.6-77.0) of detecting CIN2+ and for Multiplex HPV serology assay based on a glutathione S-transferase (GST) sensitivity was 16% (95% CI:8.45-28.6) and specificity 98% (95% CI:97-99) for detecting ICC.

Conclusion: HPV-16/18 E6 and E7 serological markers showed high specificity, but sensitivity was suboptimal for detecting cervical cancer in either population screening settings or as point–of–care screening tests.

Keywords: Cervical Cancer, Human Papillomavirus, E6 and E7 proteins antibody, sensitivity, specificity

Background

In HICs, organised and effective screening of cervical cancer using Pap smears and, more recently, HPV DNA testing (self–collected vaginal sample) have contributed to a decline in incident cervical cancer cases [121]. However, in LMICs, despite efforts to implement organised cervical cancer screening with cytology or HPV testing, coverage and uptake rates are very low, with only about 30% of women having undergone opportunistic cervical cancer screening [80,122–124]. The great benefits and minor harm of pap screening have been well documented [57,125]. However, some socio-cultural barriers (i.e. health-seeking behaviours, an embarrassment for pelvic examination by male providers, a perception that the pap smear screening process is painful, bleeding and a long turnaround time) exist around the genital examination, as shown in a recent review [126]. Therefore, conventional methods for screening cervical cancer that involve genital examination could be considered less desirable or acceptable to women. These include the more current HPV DNA or messenger ribonucleic acid (mRNA) tests that are favoured over the ThinPrep cytological test (TCT) and cytology (Pap tests) [127].

In all the screening test scenarios mentioned above, large proportions of women who screen positive for being at high risk of developing cervical cancer require recall and confirmatory diagnostic testing, then an additional recall to receive treatment. Visual inspection of the cervix with acetic acid is an alternative cervical cancer “screen and treat” screening method deemed more viable in LMIC, thus avoiding one recall step [128]. However, studies in LMIC have shown mixed results on the performance of VIA in these settings [57,128,129]. Besides, VIA has a problem of high false-positive and false-negative results and depends on the operator's experience [130,131]. As a result, the incidence rates of cervical cancer remain high in LMIC [124], and reducing the incidence

rates in these settings remains challenging. Hence, a novel efficient screening tool that is less invasive and more acceptable to women is needed.

In May 2018, the General Director of the WHO made a global call for action to eliminate cervical cancer using novel technologies to identify premalignant tumours at the earliest stage [132,133]. The 90-70-90 global cervical cancer elimination strategies call for 90% of girls to be fully vaccinated by the HPV vaccine by the age of 15 years, 70% of women should have screened with high-throughput tests by the age of 35 years and again by the age of 45 years, and 90% of women diagnosed with cervical cancer disease to be on treatment by the year 2030 [132].

One of the promising strategies is the use of serum profiling antibodies to HPV-16 and -18 early (E) 6 and E7 oncoproteins as biomarkers for the early detection of invasive cervical cancer (ICC) [134]. The E6 protein binds to the E3 ubiquitin ligase E6-associated protein (E6AP) and to the tumour suppressor protein p53, which controls the cell growth process and apoptosis [27]. The presence of E7 in human cells prevents phosphorylation of the retinoblastoma (Rb) protein leading to uncontrolled cell proliferation [26]. Out of the seven early proteins, both E6/E7 oncoproteins are involved in transforming cells, mitosis and immortalising cervical cancer cells [135]. They have shown the strongest associations with cervical cancer [136–139]. Since several antibody-based finger prick and point-of-care tests are now readily available for screening other infectious agents such as HIV [140], which have a short turnaround time (less than 1 hour), a yet-to-be-developed rapid antibody-based HPV finger prick screening test for detecting cervical cancer may be more acceptable than conventional cervical cancer screening methodologies requiring a genital examination. If such a serological test can be carried out cheaply and has a rapid turnaround time, it could save time and avoid repeat visits [141].

However, the value of hrHPV E6 and E7 serology as an alternative, less-invasive point-of-care screening tool for detecting at-risk individuals has not been thoroughly investigated. In addition, globally, there are no currently developed and USA Food and Drug Administration (FDA)-approved serological markers screening tests for cervical cancer in the general population. Studies from the early 1990s [142,143] showed some potential with sensitivities around 27- 41% and specificities around 99-100%. Therefore, we performed a systematic review to assess the utility of HPV-antibodies, especially of hrHPV-16/18 E6 and E7 serological markers, in detecting cervical cancer and cervical intraepithelial neoplasia (CIN) 2/3.

3.2. Methods

This systematic review and meta-analysis were registered and published with PROSPERO (CRD42020206036). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [144] guidelines were followed to evaluate the usefulness of HPV-16/18 E6 and E7 serological markers in detecting CIN2/3 and cervical cancer.

3.2.1. Search strategies

The search for studies was done in the following electronic databases from 2010 to 2020; PubMed and Embase (although most of the articles for this study were found in PubMed). Using the following search words “HPV”, “E6 and E7 protein”, “CIN2”, CIN3”, and “cervical cancer”. We did not include High grade squamous intraepithelial lesion (HSIL) in the search terms because HSIL is based on cytology while CIN2/3 are based on histology, which incorporates HSIL. Complete copies of the text of relevant published articles were obtained and assessed for inclusion. Additional searches were done on the references of the initial studies. Our review focused on HPV-16 and -18 because they are the most common serotypes, and they are found in 72% of cervical cancer [67].

3.2.2. Criteria for study selection

The study selection involved exporting all identified studies (N=69) combined in Mendeley into Covidence (https://www.covidence.org/). Covidence is a useful web tool for screening and selecting studies for systematic reviews. The two independent reviewers (MGS and TR) screened the

full texts for accuracy and consistency. Disagreements were resolved through discussion; any unresolved disagreements were resolved by the third reviewer (MM).

3.2.3. Inclusion

Studies were selected if they were in English, cross-sectional, retrospective case-control or prospective cohort (or nested case-control). Cases were defined as invasive cervical cancer or CIN 2/3. We used author-defined definitions of controls, broadly using similarly aged women without HPV-related conditions. Diagnostic accuracy-test studies included the HPV-16/18 E6 and E7 serological markers compared to a cyto- or histopathology reference standard.

3.2.4. Exclusion

Studies with a sample size of less than 200 could overestimate the effect size [145] (of the small studies excluded, some used an HPV E6/E7 mRNA marker, and the others used a urine Trovagene HPV test), conference papers, abstracts only and reviews were excluded. In addition, any study assessing the presence of HPV types in ICC or CIN2/3. Given regular improvements in serological techniques, studies were excluded if published before 2010. We excluded only laboratory-based studies (which did not include controls) and those studies that focused on CIN1 (Wrong outcome). Any studies presenting HPV-16/18 E6 and E7 DNA within samples of the cervical biopsy were not included because we aimed to assess the utility of anti-HPV-antibodies, especially of hrHPV-16/18 E6 and E7 in the serum of patients with ICC or CIN2/3. We further excluded papers not on serological testing, i.e. the Onco E6 test, because it detects the protein itself. In contrast, the HPV

E6/E7 mRNA tests detect the RNA encoding for the oncoproteins. Our study focused on the test accuracy of HPVE6 and E7 oncoprotein antibodies.

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3.2.5. Participants

The population of interest were women with cervical cancer and CIN2+. Studies were included if they reported women with cytology/histology-confirmed cervical cancer or high-risk lesions (e.g. CIN2 and CIN3) compared to women with the normal cervical tissue.

3.2.6. Index test

The index test was HPV-16/18 E6 and E7 and E4 proteins serological markers from the serum of the participants. The threshold determination for positive or negative results was based on cut-off test values proposed by the manufacturer or the study authors based on validated cut-off points.

3.2.7. Reference standard

The reference test was histopathologic confirmation of cervical cancer or CIN2+ in tissue in paraffin-embedded sections or cytological confirmation of CIN2/3 or cervical cancer. Methods of diagnosis were noted.

3.2.8. Study Outcome

The study's outcome was sensitivity, specificity and Diagnostic Odds Ratios (DOR) for CIN2/3 and cervical cancer.

3.2.9. Data extraction

The two reviewers independently extracted data (MGS and TR) for all the papers meeting inclusion and exclusion criteria. The following information was extracted from the eligible studies: author, year of publication, study location; sample size; the number of cases and controls, cut-offs and test manufacturer. We extracted percentages of HPV-16/18 E6 and E7 (seroprevalence data) from pre-cancers and cervical cancer cases and controls according to pre-defined cut-off values. We then extracted frequencies for true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN) for HPV-16 (E6 and E7) or HPV-18 (E6 and E7) antibodies. In some of the studies, more than one HPV-type antigen was assessed on the same individuals participating in the study. In such cases, each serological biomarker was considered an independent study when extracting the data.

3.2.10. Study Quality assessment

The quality of the studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [146]. Quality assessments of the studies were based on: a selection of participants' characteristics; the number of included participants with cervical cancer or CIN2+; description of the index test (HPV-16/18 E6 and E7), serological markers and the reference test

(histology or cytology). The studies were grouped into high-quality, low-quality and unclear. Any disagreements were resolved through discussion with the third reviewer (MM).

3.2.11.Statistical analyses

To achieve our objective, we performed a descriptive analysis of different serological markers by calculating the sensitivity and specificity, using the TP, FP, TN and FN results and their 95% Confidence Intervals (CI). Random-effects models for meta-analysis were performed to quantify pooled effects (Figure S2), and Cochrane's Q statistic and I^2 tests were used to examine heterogeneity. In the primary studies, Cases and Controls were matched by age.

Secondly, we assessed the DOR for HPV-16/18 E6 and E7 and other E oncoproteins (E4) antibody seropositivity and CIN2/3 and cervical cancer from different studies. Other studies have suggested that E4 plays an essential role in the detection of cervical cancer when used in combination with other markers [147]. We pooled the sensitivity and specificity for HPV-16/18 E6 and E7 serological markers to detect CIN2/3 and cervical cancer. Different serological screening tests ELISA, Multiplex HPV serology assay based on a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads (Multiplex HPV serology immunofluorescent assay) and immuno-enzymatic assay (Slot Blot)) were performed for comparison of pooled effects and heterogeneity. A bivariate random effect model [148] was applied using "Metandi" and "Midas" in STATA to estimate sensitivity and specificity. Metandi fits a two-level mixed logistic regression model, with independent binomial distributions for the true positives and true negatives conditional on the sensitivity and specificity in an individual study and a bivariate hierarchical normal model

for the logit transformation of sensitivity and specificity between studies. Midas calculates summary receiver operating characteristics (ROC) (Figure S3-3), summary likelihood and DOR. Hierarchical summary receiver operating characteristic (HSROC) was used to account for the correlation between sensitivity and specificity (Figure S3-4). The HSROC accounts for threshold effects and between-and within-study variability.

Statistical significance was considered at a p-value of 0.05, and all analyses were performed using STATA version 16 and the Review Manager (Version 5.3; Cochrane Collaboration, Oxford, UK). All statistical tests were two-sided.

3.3. Results

We first identified 69 records (papers) through PubMed library searches and references (Figure 3-1). We retrieved 44 potential papers after a screening of titles and abstracts. Twenty-three papers were eligible for inclusion in the systematic review. Out of twenty-three papers, only 3 papers were included with 1,550 women. A total of 11 HPV-16/18 oncoproteins antibodies (studies) were obtained from different serological test methods. Two-thirds of the studies were case-control designs, and one study was a prospective diagnostic accuracy study (Table 3-1). Assessment of the methodological quality of the individual studies was done using QUADAS-2 (Figure S3-1).

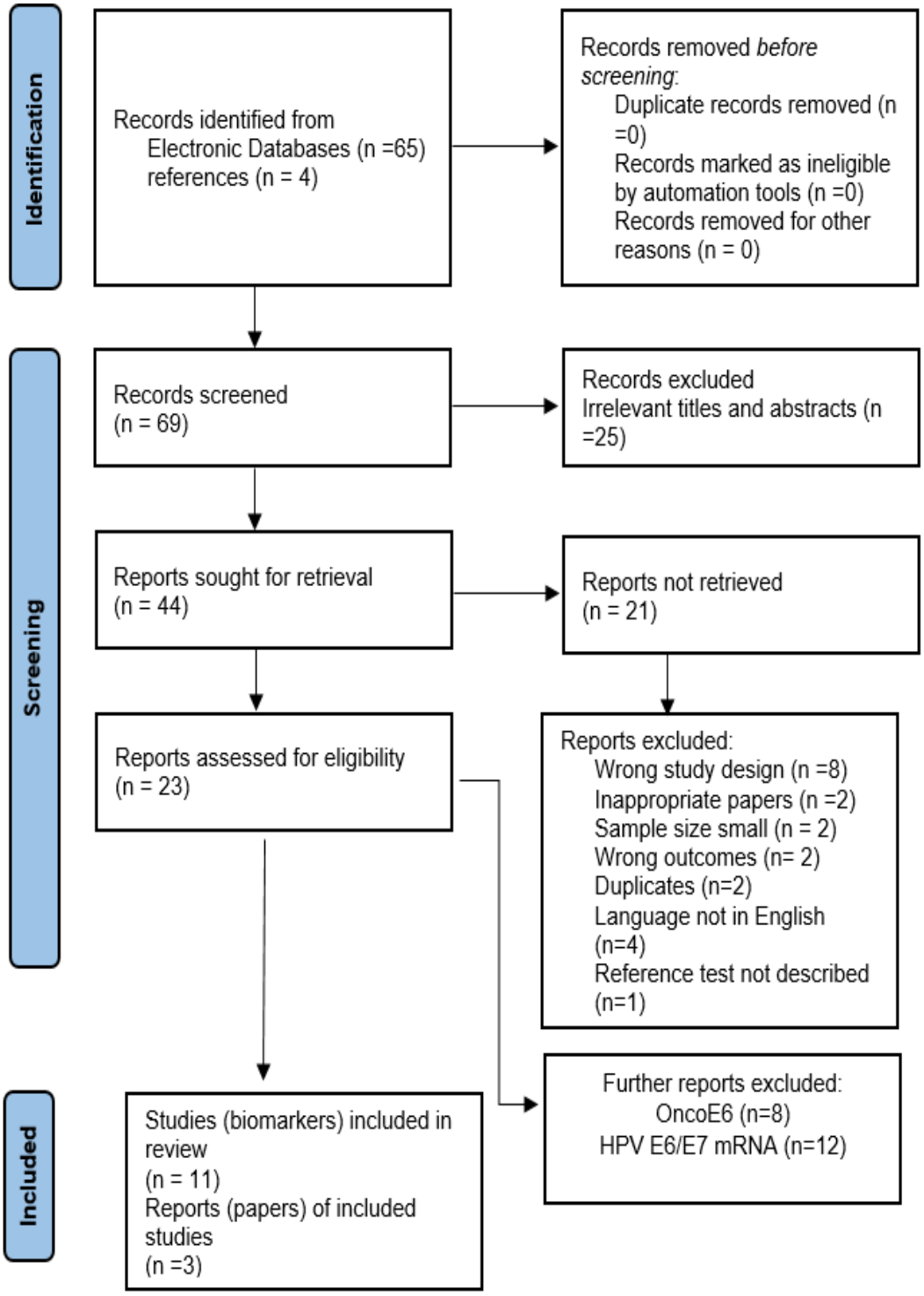


Figure 3-1: The flow of studies in the systematic review of HPV-16 and -18 E6/E7 serological markers

Table 3-1: Summary of study characteristics

No	1st Author and year	Study reference number	Country	Study design	Index test	Total	Median Age	CIN2 + n(%)	ICC n(%)
HPV-16 and -18 E6/E7 antibodies									
1	Salazar-Piña 2016	d1	Mexico	Diagnostic test accuracy study	HPV-16 E4 and E7 immunoenzymatic assay (Slot Blot)	485	40	106 (29.9)	15 (3.1)
2	Jin 2018	d2	South Korea	Case-control study	HPV E6 and E7 (ELISA)	249	44.3	144 (57.8)	-
3	Combes 2014	d3	Algeria and India	Case-control study	HPV-16 L1, E1, E2, E4, E6, and E7 (multiplex immunofluorescent HPV serology assay)	816	50.9	-	307(37.6)

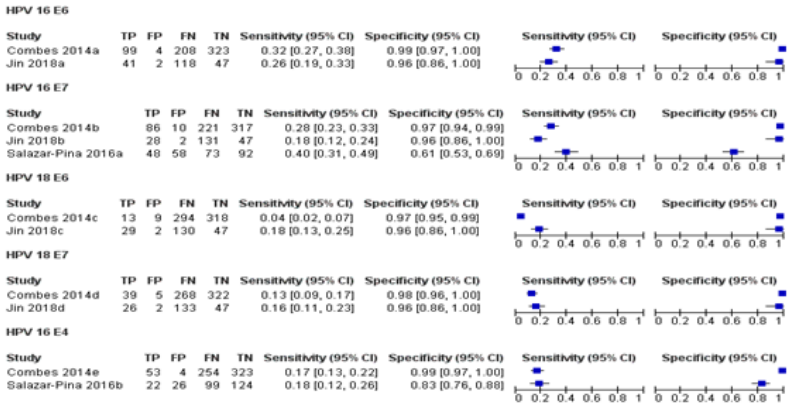
Table 3-2: True positive, False positive, False negative and True negative of different tests for detection of CIN2+ and ICC

No	Study id (1st Author year)	Study reference number	CIN2, CIN3 or Cervical cancer)	cut-off (threshold)	Test (manufacturer)	marker name (index test)	full name	TP	FP	FN	TN
1	Salazar-Piña 2016	d1a	CIN2+	2 AU/mL	Qiagen	HPV-16 E7 (Slot Blot)	HPV-16 E7 antibodies immunoenzymatic assay (Slot Blot)	48	58	73	92
	Salazar-Piña 2016	d1b	CIN2+	7 AU/mL	Qiagen	HPV-16 E4 (Slot Blot)	HPV-16 E4 antibodies immunoenzymatic assay (Slot Blot)	22	26	99	124
2	Jin 2018	d2a	CIN2+	N/A	Greiner Bio-One	HPV-16 E6 ((ELISA)	Enzyme-linked immunosorbent assay	41	2	118	47
	Jin 2018	d2b	CIN2+	N/A	Greiner Bio-One	HPV-16 E7 ((ELISA)	Enzyme-linked immunosorbent assay	28	2	131	47
	Jin 2018	d2c	CIN2+	N/A	Greiner Bio-One	HPV-18 E6 ((ELISA)	Enzyme-linked immunosorbent assay	29	2	130	47
	Jin 2018	d2d	CIN2+	N/A	Greiner Bio-One	HPV-18 E7 ((ELISA)	Enzyme-linked immunosorbent assay	26	2	133	47
3	Combes 2014	d3a	ICC	394 to 714 MFI	Luminex Corp.	HPV-16E6 (Multiplex)	Multiplex HPV serology immunofluorescent assay	99	4	208	323
	Combes 2014	d3b	ICC	395 to 714 MFI	Luminex Corp.	HPV-16E7 (Multiplex)	Multiplex HPV serology immunofluorescent assay	86	10	221	317

No	Study id (1st Author year)	Study reference number	CIN2, CIN3 or Cervical cancer)	cut-off (threshold)	Test (manufacturer)	marker name (index test)	full name	TP	FP	FN	TN
	Combes 2014	d3c	ICC	396 to 714 MFI	Luminex Corp.	HPV-18E6 (Multiplex)	Multiplex HPV serology immunofluorescent assay	13	9	294	318
	Combes 2014	d3d	ICC	397 to 714 MFI	Luminex Corp.	HPV-18E7 (Multiplex)	Multiplex HPV serology immunofluorescent assay	39	5	268	322
	Combes 2014	d3e	ICC	394 to 714 MFI	Luminex Corp.	HPV-16 E4 (Multiplex)	Multiplex HPV serology immunofluorescent assay	53	4	254	323

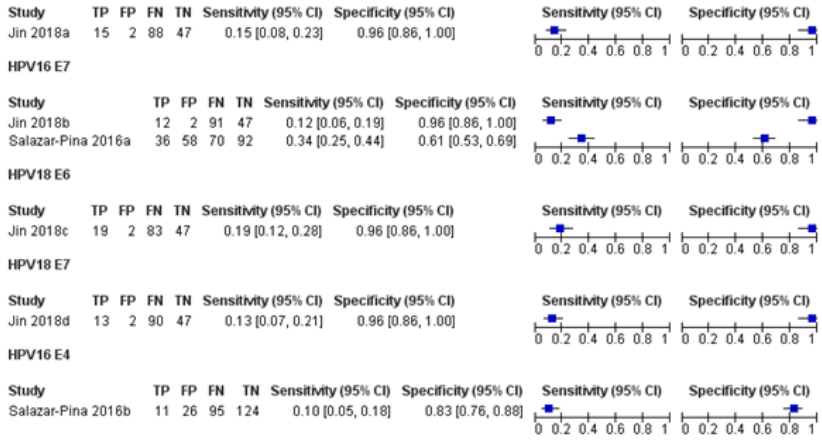
There was a considerable degree of heterogeneity for sensitivity between the studies for combined CIN2/3 and ICC, which ranged between 0-0.40, while specificity was very good for most of the biomarkers. Thus, we did not pool the studies (Figures: 3-2 A-C). For the stratified analysis by tumour stage, sensitivity was very low for CIN2/3, which ranged between 0- 0.20 for the biomarkers, while specificity was above 0.90 for most of the biomarkers. For invasive cervical cancer, the sensitivity ranged between 0.20-0.40 and specificity between 0.96-0.97.

A



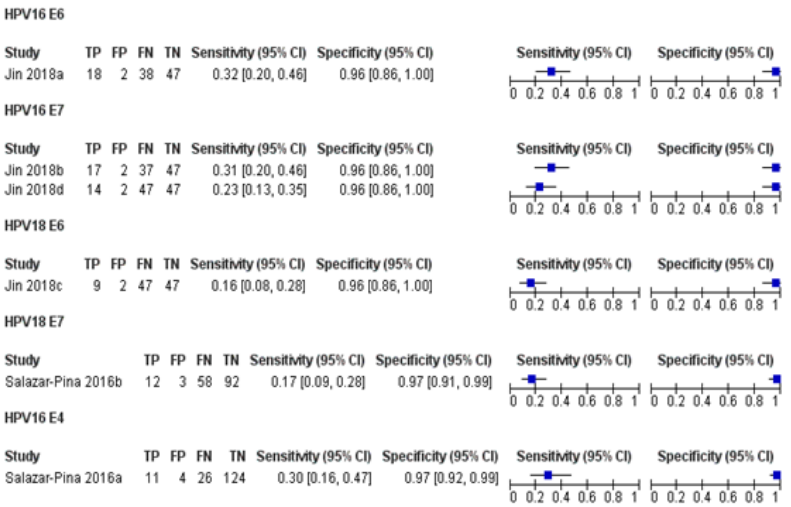
Forest plots of sensitivity and specificity for different serological marker studies in detection of CIN2/3 and ICC

B



Forest plots of sensitivity and specificity for different serological marker studies in detection of CIN2/3

C



Forest plots of sensitivity and specificity for different serological marker studies in detection of Invasive Cervical Cancer

Figure 3-2: Forest plots of sensitivity and specificity for different serological marker studies in detecting CIN2/3 and ICC. Figure 3-2A: Forest plots of sensitivity and specificity in detecting CIN2/3 and ICC. Figure 3-2B: Forest plots of sensitivity and specificity in detecting CIN2/3. Figure 3-2C: Forest plots of sensitivity and specificity in detecting ICC.

Different assays were used to characterise the association between HPV E6 and E7 antibodies and cervical cancer. In the studies that used ELISA, the pooled sensitivity was 18% (95% CI:15-21), and specificity was 96% (95% CI:92-98) (Table 3-3). For the studies that used Multiplex immuno-fluorescent assay, the pooled sensitivity was 16% (95% CI:8.45-28.6), and specificity was 98% (95% CI:97-99) (Table 3-3). For the studies that used immunoenzymatic assay (Slot Blot), the pooled sensitivity was 28.9% (23.3-35.1), and specificity was 72% (95% CI:66.6-77.0) (Table 3-3). The multiplex immunofluorescent assay had a DOR of 9.72 (95% CI:3.95-23.93), the ELISA had a DOR of (5.00; 95% CI: 2-11) and the immunoenzymatic assay (DOR=1.05; 95%CI:0.72-1.52.

Table 3-3: Bivariate meta-analysis of different HPV-16 and -18 serological markers: Pooled sensitivity and specificity by assay type

	Marker name	Study reference number	Number of studies	Endpoint	Sensitivity (95%CI)	Specificity (95% CI)	DOR (95%CI)
HPV Serology (ELISA)	HPV-16 and -18 (E6 and E7)	d2a, d2b, d2c, d2d	4	CIN2+	18.0 (15.0-21.0)	96.0 (92.0-98.0)	5.00 (2.00-11.00)
HPV serology (Multiplex immunofluo-rescent assay)	HPV-16 and -18 E6, E7 and E4	d3a, d3b, d3c, d3d, d3e	5	ICC	16.0 (8.45-28.6)	98.0 (97.0-99.0)	9.72 (3.95-23.93)
HPV serology (immunoenzymatic assay (Slot Blot))	HPV-16 E7 and E4	d1a, d1b	2	CIN2+	28.9 (23.3-35.1)	72.0 (66.6-77.0)	1.05 (0.72-1.52)

Across the studies, HPV antibody sensitivity was low, ranging from 4%-40%, while specificity was high, ranging from 61% to 99%. From the HSROC curve, HPV-16 E6 antibody presence appears to be a better marker than other markers (Figure 3-3).

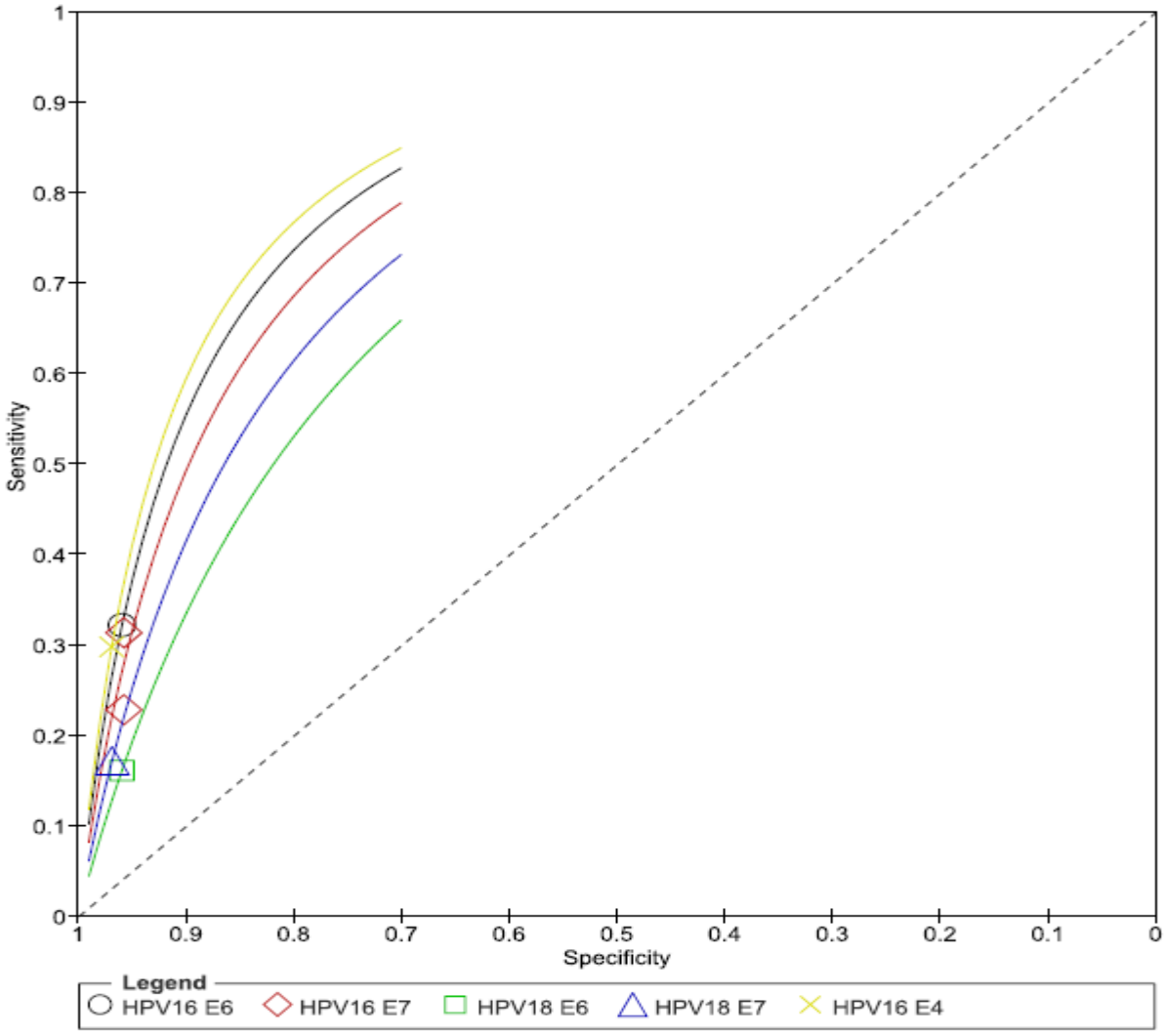


Figure 3-3:Summary of the HSROC plots of sensitivity and specificity for HPV-16 and -18 proteins serological markers.

3.4. Discussion

Antibodies to HPV oncoproteins have been suggested as important serological markers for cervical cancer. Our systematic review and meta-analysis focused on the sensitivity and specificity of HPV-16/18 early oncoproteins antibodies. Our main findings suggest that HPV-16 and -18 E6 and E7 antibodies have high specificity but low sensitivity to detect cases with cervical cancer or CIN2/3 precancerous lesions. Another key finding is that there is scant literature on E6/E7 as a biomarker for ICC and CIN2/3.

We found that HPV-16 and -18 E6 and E7 antibodies have higher specificity but lower sensitivity. Our estimates are similar to the pre-2010 estimates of Sun et al. [149] in Brazil, where they reported a specificity of 99.5% and sensitivity of 40.7% for HPV-16 E6 or E7 (higher antibody titers). Similarly, in Japan, the findings of Sasagawa et al. [143], using ELISA test with E6 or E7 proteins as antigens, had first shown in 1992 that 27% of cervical cancer and 19% of CIN3 were positive for anti-HPV-16 E6 antibodies, while 33% in cervical cancer and 8% in CIN3 were positive for anti-HPV-16 E7. Therefore, low sensitivity in these serological tests may represent a lower immune response against HPV-16/18 E6 or E7 protein in many patients with these malignant lesions. Another explanation for the low sensitivity of these assays might be due to inadequate immune responses against HPV-16/18. The serological responses to HPV-16/18 E6 and E7 are more likely surrogate markers for cytotoxic immune responses by cytotoxic T lymphocyte (CTL) targeting these proteins. Such CTLs function to kill CIN or cancer cells positive with HPV-16 or 18 [150]. In addition, our findings showed that the HPV-16 E6 antibody is a relatively better serological marker than the other biomarkers. Similarly, a recent systematic review and meta-analysis on

blood-based biomarkers of HPV-associated cancer found HPV-16 E6 antibodies to be a better serology marker [151]. Although HPV-16 E6 has been demonstrated to be a better biomarker, its sensitivity is still low for it to be used as a screening tool. Additional studies are needed for its validation with other biomarkers [151].

HPV-16 E6 and E7 antibodies have been associated with the tumour stage of cervical cancer [150,152–155]. Our study found that the sensitivity for CIN2/3 was lower than for ICC. Our findings agree with Park et al. [156], which reported that HPV-16 E6 and E7 antibodies seropositivity is associated with tumours by Stages. Their findings showed that seropositivity for HPV-16 E6 was 51% and 38% for HPV-16 E7 in Stage II and HPV-16 E6 (69%) and HPV-16 E7 (56%) for Stage III/IV. In addition, in Squamous carcinoma, seropositivity was 54% for HPV-16 E6 and 32% for HPV-16 E7. However, a Radioimmunoprecipitation Assay (RIPA) was used, making comparison difficult between assays. Similarly, in Korea, Chee et al. [150], using RIPA, found that HPV-16 E6 and E7 antibodies were associated with the clinical stage of the tumour for HPV-16 E6(CIN (4.2%), Stage I (12.5%),. Stage IIa (35.7%), Stage IIb (100%), Stage III (100%)) and HPV-16 E7 (CIN (4.2%), Stage I (43.8%), Stage IIa (57.1%), Stage IIb (60%), Stage III (100%)).

The strengths of our systematic review and meta-analysis include applying the standard systematic review protocol, using Covidence software to screen the studies, involvement of second and third reviewers at all screening stages, and using QUADAS-2 to assess the quality of the studies' methodology. Our study had several limitations: After all the exclusions, only a few studies were included that evaluated the antibodies against HPV E6 and E7 oncoproteins for detecting CIN2/3 and cervical cancer. There was a high level of heterogeneity in the included studies. Therefore it was impossible to have pooled estimates of all the studies. Due to the different assays used, we could

not pool all the studies together. In addition, due to the selection bias that comes with the case-control design, we could not pool the studies together [157]. Our systematic review and meta-analysis only covered studies published in the last 10 years and might have missed studies published in other languages or not appearing during the literature search.

This systematic review and meta-analysis focused on the utility (for example, sensitivity and specificity) of HPV-antibodies, especially of HPV-16/18 E6 and E7 serological markers in detecting ICC and CIN2/3. Recently, the WHO, in their new cervical cancer guidelines, suggested HPV antibodies and oncoproteins as possible future screening tests [60]. If such a test could be incorporated into population screening using point-of-care testing of appropriately aged non-vaccinated women (e.g. 30-65 years as recommended by cervical screening guidelines) [60], it would improve screening rates, reduce losses to follow-up (people, samples and results) and ultimately lead to early detection of the disease in limited-resource settings. Individuals who have been seropositive to both HPV E6/E7 antibodies are at higher risk for cervical cancer [83], making them potentially useful biomarker candidates for early detection [30]. However, we found that evidence is still limited for HPV antibodies to be translated into an effective detection tool for cervical cancer. Further laboratory studies are required to develop more accurate HPV serological biomarkers to be successfully translated into an effective screening or point–of–care detection tool for cervical cancer.

Conclusions

Our systematic review and meta-analysis further add to the debate that HPV-16 and -18 E6 and E7 antibodies could be helpful biomarkers for cervical cancer screening. There seems to be very little improvement over time in the utility of serological markers in detecting cervical cancer or CIN3.

Although the specificity of HPV serological markers is high, their sensitivity is suboptimal for detecting cervical cancer.

Acknowledgements

We acknowledge the contributions of all authors in the ERICA_SA collaborative for their efforts in providing feedback to improve the final version of the manuscript.

Supplementary Figures

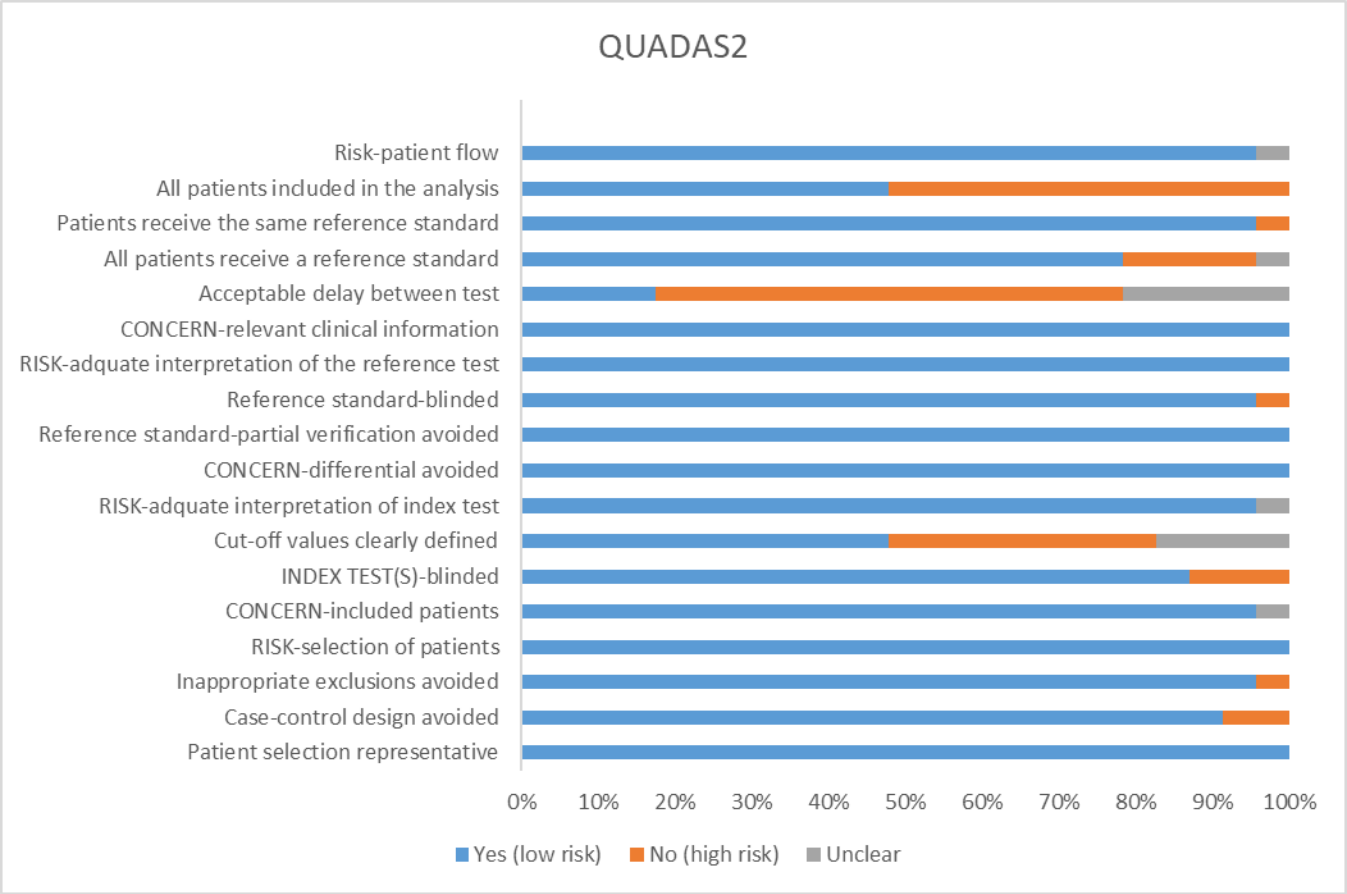


Figure S 3-1: Methodology quality assessment graph

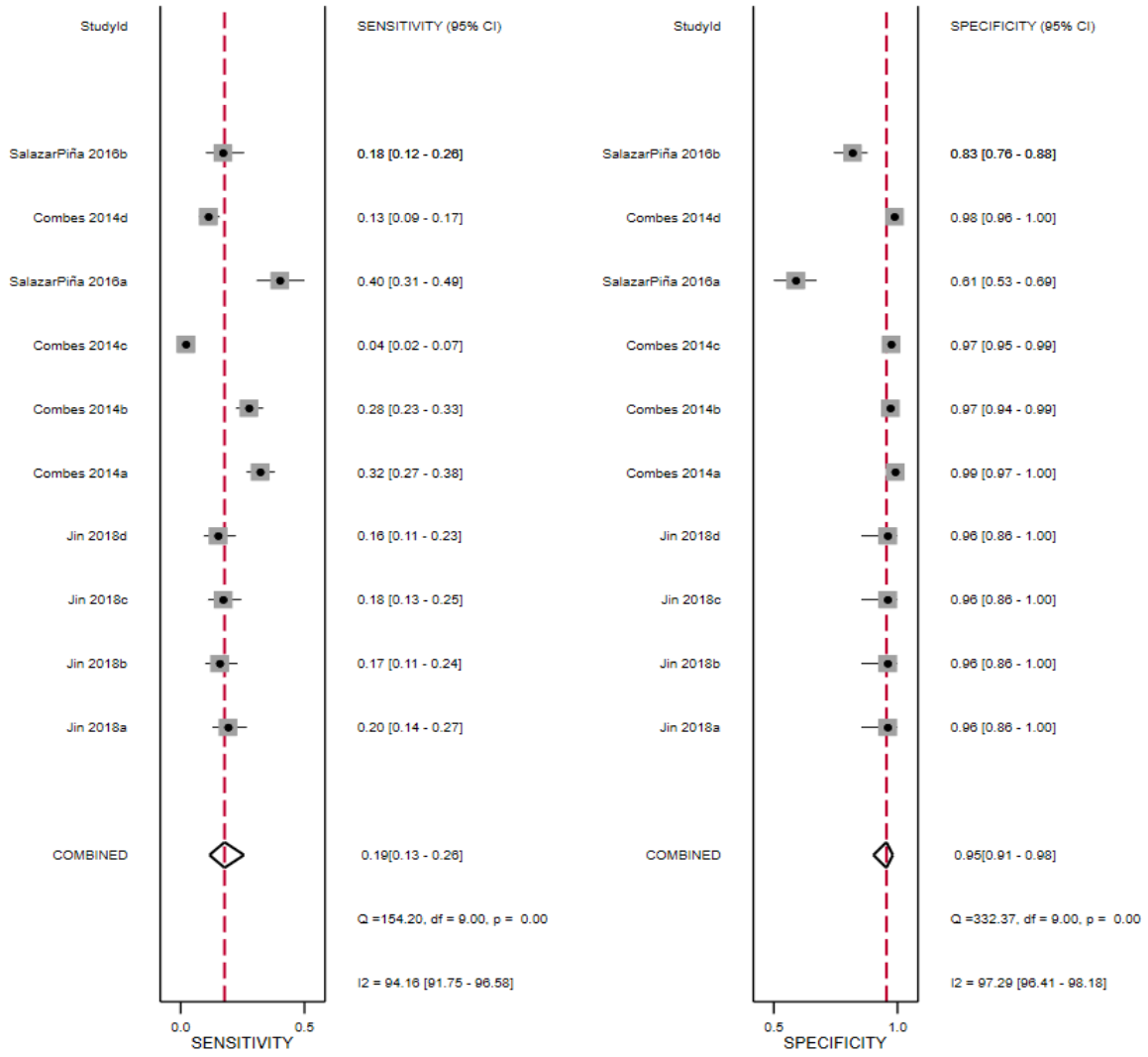


Figure S 3-2: Pooled Sensitivity and Specificity for HPV antibodies

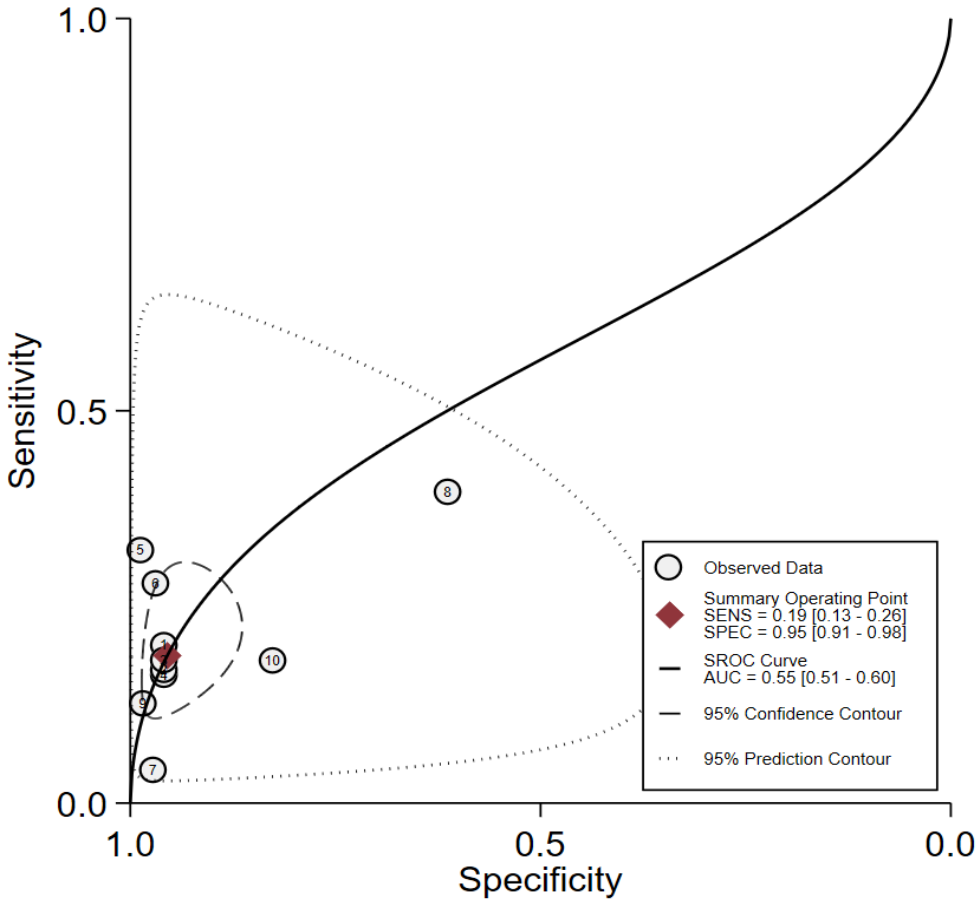


Figure S 3-3: SROC curve in HPV-antibody tests

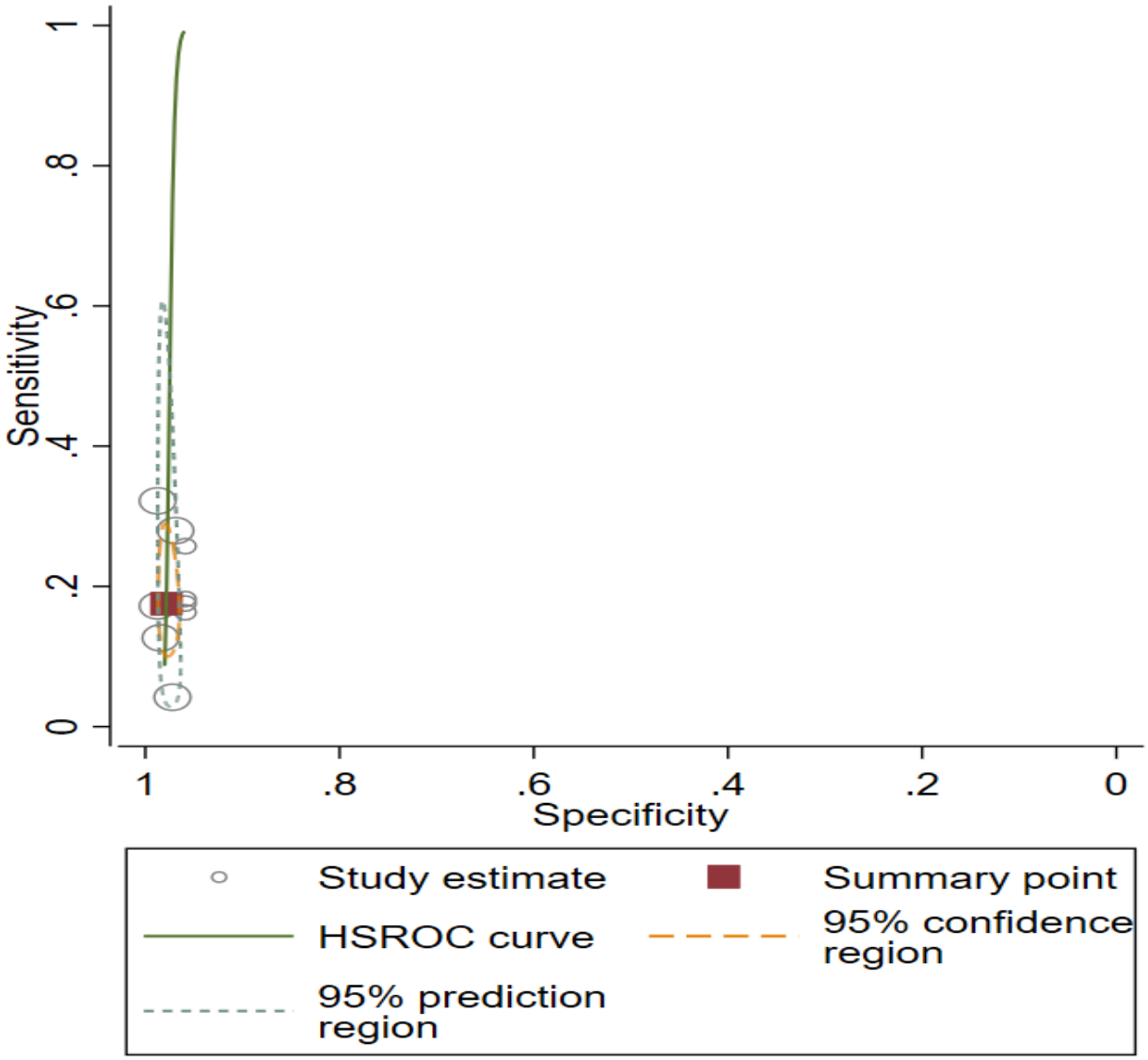


Figure S 3-4: HSROC curve showing heterogeneity in HPV-antibody study (Salazar-Piña study not included (Metandi was failing to run due to variations in the studies)

4.0. Chapter Four: HPV types 16/18 L1 E6 and E7 proteins seropositivity and Cervical Cancer Risk in HIV-Positive and HIV-Negative Black South African Women

Authors: Mwiza Gideon Singini, Elvira Singh, Debbie Bradshaw, Wenlong Carl Chen, Melitah Motlhale, Abram Bunya Kamiza, Chantal Babb de Villiers, Mazvita Muchengeti, Christopher G. Mathew, Robert Newton, Noemi Bender, Tim Waterboer and Freddy Sitas.

Published in Infectious Agents Cancer 17, 14 (2022).

4.1. Introduction

The chapter presents results from some of the gaps that were identified in a systematic review and meta-analysis. In sum, only 11 studies comprising a total sample size of just under 1,550 measured the utility of HPV serology in detecting cervical cancer/CIN3, none of which were from Africa, and none differentiated participants by their HIV status. Using data from the largest available cancer study in Africa, (comprising 1346 women with and 2532 without cervical cancer) a case-control analysis was performed that assessed, in a more reliable way, seropositivity to HPV types 16 and -18 E6 and E7 oncoprotein serological markers and cervical cancer risk in HIV-Positive and HIV-negative Black South African women in a local setting.

This chapter addresses the second and the third out of the four objectives of the PhD thesis. The chapter shows that HPV-16 and -18 L1, E6 and E7 proteins are associated with cervical cancer among both HIV-Positive and HIV-negative Black South African women. This analysis confirmed that HPV-16 and -18 E6/E7 antibodies have low sensitivity and higher specificity, but the sensitivity was better than in previous reports, almost similar to what is observed with Pap test screening. With strong caveats, this may be a useful tool for future, less invasive screening programs. The paper was published in Infectious Agents and Cancer.

Abstract

Background
In populations with high HIV-coinfection rates, the relationship between HPV-16 and -18 (L1, E6 and E7) antibodies and cervical cancer is still uncertain. We measured the association between seropositivity to HPV (L1, E6 and E7) proteins and cervical cancer among Black South African women with and without HIV co-infection.

Methods
We used questionnaire data and serum collected from consecutively recruited patients with newly diagnosed cancer from the JCS from 1,346 cervical cancer cases and 2,532 controls (diagnosed with other non-infection-related cancers). Seropositivity to HPV proteins was measured using a multiplex serological assay based on recombinant glutathione S-transferase (GST) fusion proteins. We measured associations between their presence and cervical cancer using unconditional logistic regression models and evaluated the sensitivity and specificity of these HPV biomarkers.

Results
Among controls, HIV-negative women from rural areas compared to urban had significantly higher HPV seroprevalence, HPV-16 E7 (8.6% vs 3.7%) and HPV-18 E7 (7.9% vs 2.0%). HPV-16 E6 and E7 antibodies were positively associated with cervical cancer in HIV-positive (Adjusted Odds Ratio (AOR)=33;95% CI:10-107) and HIV-negative women (AOR=97;95% CI:46-203). In HIV-positive women, HPV E6/E7 antibodies had low sensitivity (43.0%) and high specificity (90.6%) for cervical cancer detection. In HIV-negative women, HPV E6/E7 antibodies sensitivity was 70.6%, and specificity was 89.7%.

Conclusions
Our data show that HPV (L1, E6 and E7) antibodies are associated with cervical cancer in HIV-positive and HIV-negative women. Nonetheless, HIV-positive plays an important role in developing cervical cancer.

Keywords: Cervical Cancer, Human Papillomavirus, E6 and E7, L1 proteins, seropositivity, South Africa

Background

Globally, in 2018, about 70% of cervical cancers were attributable to hrHPV-16 and -18 [67]. In South Africa, HPV-16 and -18 are important causes of cervical cancer and are included in the currently available HPV vaccine.

The HPV early (E6 and E7) oncoproteins and the late (L1) protein are encoded by the HPV genome. HPV oncoproteins play an important role in cervical cancer tumorigeneses [158]. Humoral immune response against major capsid late protein (L1) is generated during infection with HPV, which is a marker of past or present infection [26]. Furthermore, integration of the HPV genome in the host cell results in the overexpression of E6 and E7 oncoproteins, which are involved in the transformation and progression of cervical and other HPV-related cancers [159]. Different serological assays have been used to screen HPV-16 and -18 (E6 and E7) [31,160–162] oncoprotein antibodies. Serological markers for HPV antibodies provide useful data about past HPV infections and their relationship to cervical cancer [86,91].

Previous studies have shown that HPV types 16 and -18 L1, E6 and E7 antibody proteins are associated with cervical cancer [31,161,163–165]; however, with varying degrees of strength of association. For example, Zumbach et al. [31] utilized an ELISA in a case-control study from Russia. They reported odds ratios (ORs) for cervical cancer of 64.4 (95% CI: 3.8-1085) for HPV-16 E6 and 4.9 (95% CI: 1.3-18.7) for HPV-16 E7. In another case-control study from Algeria and India, Combes et al. [91] used a multiplex immunofluorescent HPV serology assay. They reported ORs for cervical cancer of 37.1 (95% CI: 13.4-103) for HPV-16 E6, 12.5 (95% CI: 6.3-24.8) for

HPV-16 E7 and 5.9 (95% CI:3.1-11.1) for HPV-16 L1. Earlier work on the JCS from 946 HIV-negative women with cervical cancer and 1,342 controls using an ELISA assay and different cut-offs found ORs between moderate and high HPV-16L1 seropositivity of 1.5 (95% CI:1.2-1.9) and 2.4 (95% CI:1.9-3.0) [35]. Thus, multiple serologic methods have been used to characterize the relationship between cervical cancer incidence and HPV proteins.

Less is known about the role of hrHPV oncoproteins among cervical cancer patients in Black African HIV-positive and HIV-negative women. In South Africa, despite high HIV prevalence (24.1%) among Black South African women [166], studies on HPV-16 and -18 (E6 and E7) and late (L1) seropositivity and the risk of cervical cancer are still lacking. We, therefore, assessed the seropositivity of HPV-16 and -18 (E6 and E7) and L1 antibodies among cervical cancer cases and infection-unrelated cancer controls using a multiplex HPV serology assay [37] and estimated their association with cervical cancer risk among HIV-positive and HIV-negative women.

4.2. Methods

4.2.1. Setting and participants

The study population were participants recruited into the JCS between 1995 and 2016. The aims of JCS included measuring the relative importance of known and emerging risk factors for cancer in a Black African population in Johannesburg, South Africa. The details of the JCS have been described elsewhere [33]. Briefly, the JCS collected serum samples from about 26,000 consecutive consenting Black South African patients with newly diagnosed cancers (>90% histopathology confirmed), referred to the medical oncology and radiation therapy wards of Charlotte Maxeke Johannesburg Academic Hospital and associated referral hospitals and clinics. Self-reported data on demographics and key lifestyle risk factors were collected using a structured questionnaire.

The JCS also collected venous blood in one serum separation tube (SST) and one Ethylenediaminetetraacetic acid (EDTA) tube. SST serum was separated from whole blood within two days of sample collection, and the sample was divided into a maximum of 4 aliquots. HIV testing was done using the Vironostika HIV Ag/Ab kit [38]. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number: M200252).

4.2.2. Cases and Controls

The JCS is amenable to a case-control design and analysis by selecting controls that are unrelated to the exposures of interest. The current study was restricted to women aged 25 to 54 because many older women did not have serology samples available. Cases were 1,346 women with newly

diagnosed cervical cancer (ICD-10=C53). Controls were 2,532 women with newly diagnosed infection unrelated cancers (breast (C50) (n=1,953), colon (C18-20) (n=197), oesophagus (C15) (n=48), endometrium (C54-55) (n=75), lung (C33-34) (n=76), pancreas (C25) (n=21) and minor cancer types (n=162) (Table S4-1)). Figure 4-1 outlines the criteria used to select cases and controls.

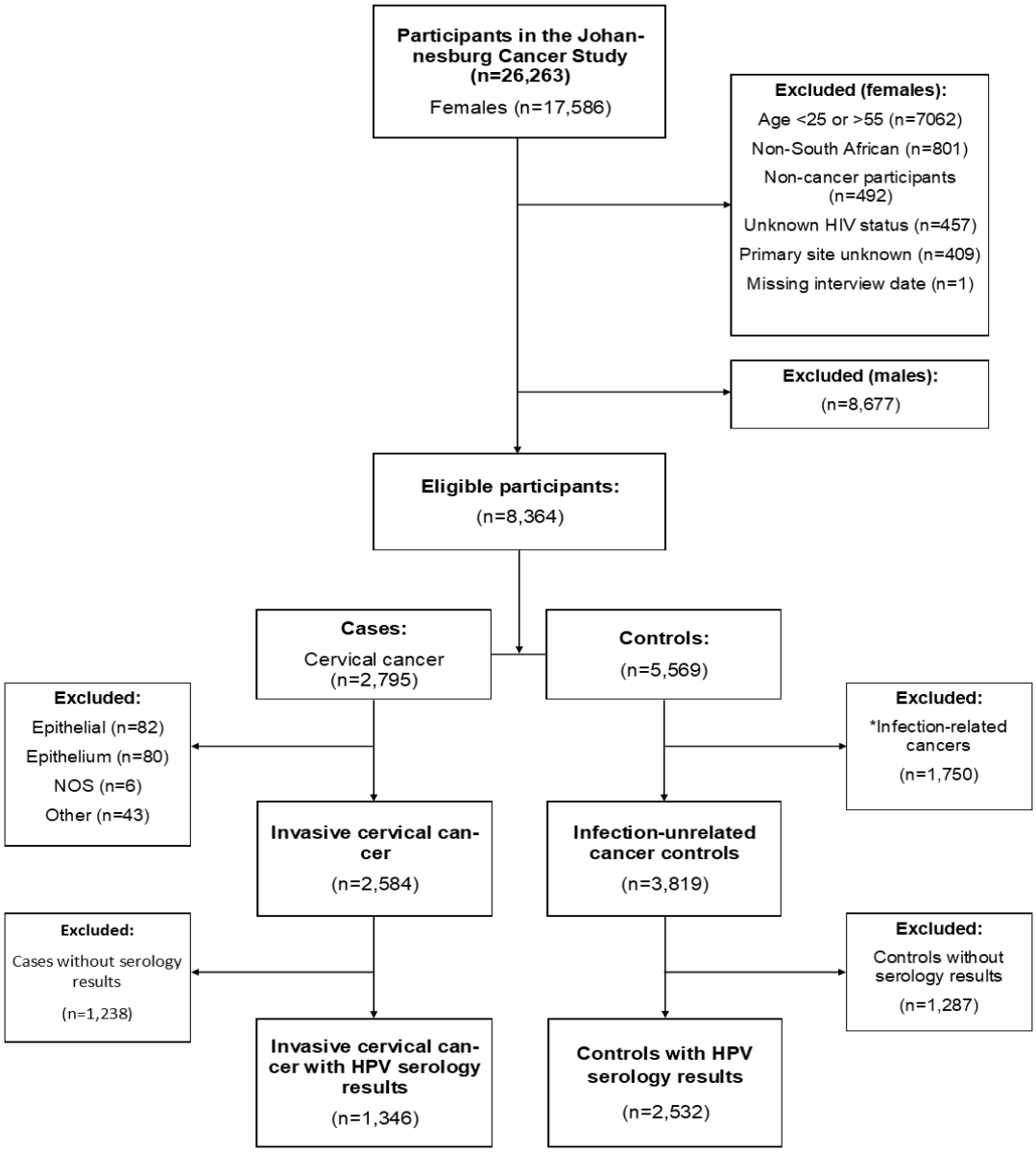


Figure 4-1: Flow chart of the case-control selection for the subject with HPV-serology results among women aged 25-54 years in the JCS.

4.2.3. Serology data and laboratory methods

Serum aliquots were stored at -25℃, and one aliquot was shipped on dry ice to the German Cancer Research Centre (Deutsches Krebsforschungszentrum (DKFZ)) in Heidelberg for antibody testing for HPV-16 and- 18 (L1, E6 and E7) using a multiplex serological assay. This was based on a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads on a Luminex platform [37]. The final net (bead and GST background subtracted) Median Fluorescence Intensity (MFI) values of all samples were analysed at a dilution of 1:1000. Previously established standard cut-offs were generated at 1:100 serum dilution [167,168] and were thus not applicable. Using the Visual Inflection Point (VIP) method [169], we defined net MFI cut-offs of 175 for HPV-16 and -18 L1, 75 for HPV-16 and -18 E6, 120 for HPV-16 E7, and 70 for HPV-18 E7. For HPV antibodies, we generated five extra binary variables to describe high-risk combinations for the presence of either E6 /E7 singly or in combination (HPV-16 E6 & E7, HPV-18 E6 & E7, HPV-16&18 E6, HPV-16 &18 E7, and HPV-16/18 E6/E7).

4.2.4. Statistical analysis

Data on demographic characteristics were summarized using frequencies and percentages for the categorical variables, medians and the interquartile range (IQR) for the continuous variable that was not normally distributed. Pearson's Chi-squared test (for categorical variables) and t-test (for age) were used to compare demographics between cases and controls. A tetrachoric correlation coefficient rank test [170], with Bonferroni adjustments (to take account of multiple comparisons), was used to analyse the correlation between each HPV-16 and -18 (L1, E6 and E7) antibody and

HIV antibody (Figure 4-2). In the control group, we calculated the seroprevalence of antibodies to HPV-16 and -18 (E6 and E7) oncoproteins and L1 protein across different demographic and life-style factors, such as age (25-34, 35-44, 45-54 years), place of residence (rural, urban), period of interview (1995-1999, 2000-2004, 2005-2009, 2010-2016), marital status (never married, married, previously married), educational level (none, primary, secondary and above), number of sexual partners (0-1, 2-5, 6+), HIV-status (negative and positive) and parity (0, 1, 2, 3, 4+). We have previously shown that cancer types unrelated to the exposure of interest resemble background population prevalence for that exposure [38,171]. We performed a test for heterogeneity on categorical variables and a score test for trends in proportions for ordinal categorical variables. On the assumption that the selected cancer types in the controls should have similar HPV seroprevalences to each other, we compared heterogeneity in seroprevalence of HPV-16 and -18 (L1, E6 and E7) antibodies across different control cancer types using the Cochran-Mantel-Haenszel test (Figure S4-1).

We used an unconditional logistic regression model to assess the association by calculating adjusted Odds Ratios (AOR) and 95% confidence intervals between HPV-16 and -18 (E6 and E7) and L1 protein seropositivity and the risk of cervical cancer. We stratified by HIV status to assess if the seroprevalence of the HPV proteins differs in HIV-positive and HIV- negative patients. We adjusted for age, education level, number of sexual partners, marital status, interview period, and place of residence. To assess whether the clinical performance of HPV-16 and -18 antibodies as a diagnostic marker for cervical cancer perform the same in HIV-positive and HIV-negative cancer patients. We calculated the sensitivity and specificity of the various HPV antibodies for detecting cervical cancer using the "diagti" command in STATA. In addition, the area under the ROC curve

(AUC) of the receiver operator characteristics was computed to compare the performance of the best combination of HPV E6 and E7 antibodies to discriminate cervical cancer from controls. All the statistical tests were computed using STATA software version 16.0 (Stata Corp, college station, Tx) and SAS v 9.4 (SAS Institute, Cary, NC). All tests were considered significant at a two-tailed alpha of 5%.

4.3. Results

Out of a total of 2,795 cervical cancer cases and 5,569 infection-unrelated cancer controls participating in the JCS, 3,878 women (1,346 cases and 2,532 controls) aged 25-54 years were included (Figure 4-1). Cases were relatively younger (Median age: 42 (IQR:37-46) compared to controls (Median age: 44 (IQR: 39-50) (p-value <0.001). At least two-thirds of both cases (63.5%) and controls (72.4%) had a secondary school education (Table 4-1).

Table 4-1:Comparison of demographic characteristics of study participants from the JCS (1995 – 2016)

Characteristics	Total (N=3,878 N (%)	Cervical cancer Cases (N=1,346) n (%)	Controls (N=2,532) n (%)	Comparison of cases and controls p-value
Age				
Median (IQR)	44 (38-48)	42 (37-46)	44 (39-50)	<0.001[#]
25-34	528 (13.6)	186 (13.8)	342 (13.5)	<0.001[$]
35-44	1,631 (42.1)	693 (51.5)	938 (37.1)	
45-54	1,719 (44.3)	467 (34.7)	1,252 (49.5)	
Period of interview				
1995-1999	255 (6.6)	133 (9.9)	122 (4.8)	<0.001
2000-2004	717 (18.5)	254 (18.9)	463 (18.3)	
2005-2009	1,431 (36.9)	538 (40.0)	893 (35.3)	
2010-2016	1,475 (38.0)	421 (31.3)	1,054 (41.6)	
Number of sexual partners				
0-1	269 (6.9)	78 (5.8)	191 (7.5)	<0.001
2-5	2,400 (61.9)	929 (69.0)	1,471 (58.1)	
6+	505 (13.0)	192 (14.3)	313 (12.4)	
Unknown	704 (18.2)	147 (10.9)	557 (22.0)	
Parity				
0	68 (1.8)	12 (0.9)	56 (2.2)	<0.001
1	632 (16.3)	174 (12.9)	458 (18.1)	
2	1,008 (26.0)	335 (24.9)	673 (26.6)	
3	922 (23.8)	325 (24.2)	597 (23.6)	
4+	1,090 (28.1)	473 (35.1)	617 (24.4)	
Missing data	158 (4.1)	17 (2.0)	131 (5.2)	
Marital Status				
Never married	1,009 (26.0)	356 (26.5)	653 (25.8)	<0.001

Married	1,941 (50.1)	682 (50.7)	1,259 (49.7)	
Previously married	919 (23.7)	303 (22.5)	616 (24.3)	
Missing data	9 (0.2)	5 (0.4)	4 (0.2)	
Place of Residence				
Rural	317 (8.2)	150 (11.1)	167 (6.6)	<0.001
Urban	3,556 (91.7)	1,195 (88.8)	2,361 (93.3)	
Missing data	5 (0.1)	1 (0.1)	4 (0.2)	
HIV-status				
Negative	2,535 (65.4)	670 (47.8)	1,865 (73.7)	<0.001
Positive	1,345 (34.6)	676 (50.2)	667 (26.3)	
Education Level				
None	278 (7.2)	128 (9.5)	150 (5.9)	0.016
Primary	903 (23.3)	358 (26.6)	545 (21.5)	
Secondary and above	2,688 (69.3)	854 (63.5)	1,834 (72.4)	
Missing data	9 (0.2)	6 (0.5)	3 (0.1)	

Controls are cancers unrelated to infection, Ever married includes widowed and divorced, IQR=Interquartile range, [#]p-value for a t-test, [$]p-value for the chi-squared test

Overall, 50.2% of women with cervical cancer were HIV positive vs 26.3% in the controls (Table 4-1). Furthermore, 13.5% of HPV-16 L1 seropositive women were also HPV-18 L1 seropositive (Additional Table 4-3).

Stratifying by HIV-status, HPV-16 E6 & E7 and HPV-18 E6 & E7 seropositivities were higher among HIV-negative women amongst both cases HPV-16 E6 (42.7%) and controls HPV-16 E6 (3.0%) as compared to HIV-positive women cases HPV-16 E6 (26.8%) and controls HPV-16 E6 (2.8%) (Figures 4-2).

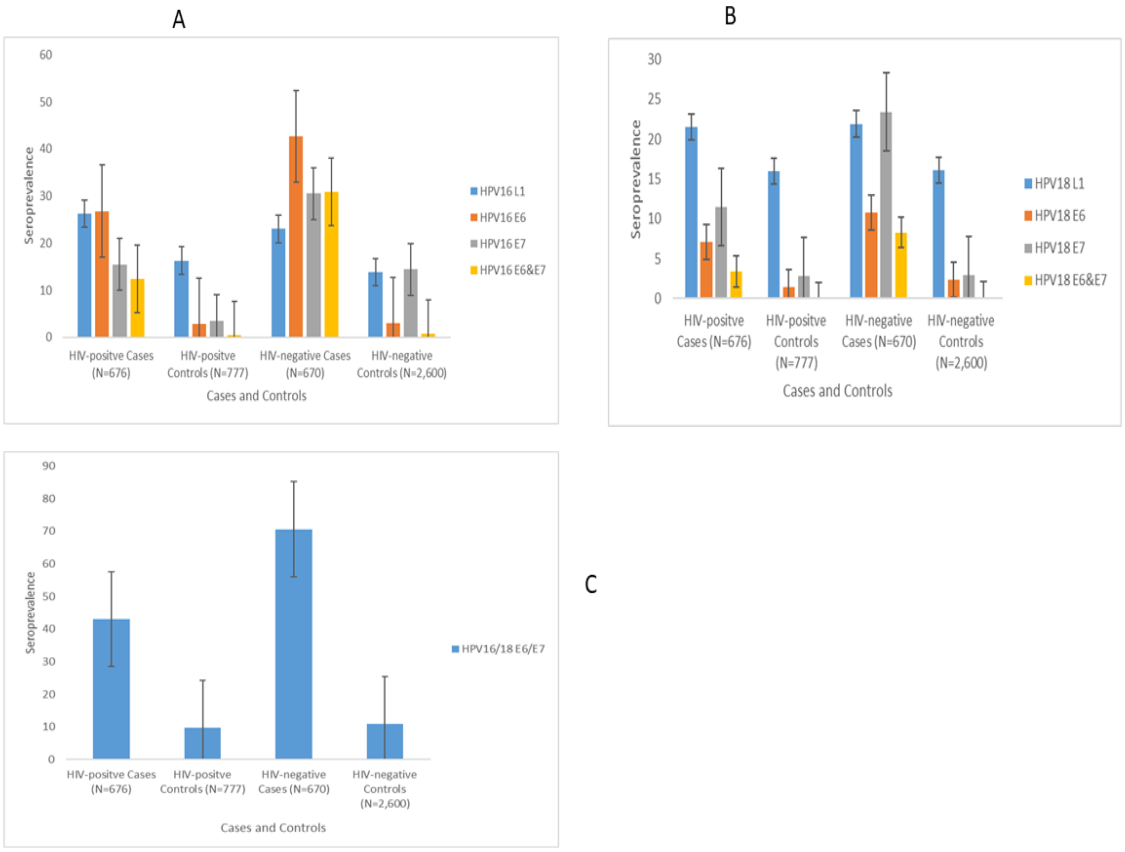


Figure 4-2: Seroprevalence of HPV-16 and -18 (L1, E6 and E7) proteins antibodies in cervical cancer cases and controls by HIV status.

Among HIV-negative controls, the overall antibody seroprevalence for HPV-16 L1 was 14.3% and 0.5% for combined HPV-16 E6 & E7. Similar observations were made for HPV-18 L1 (16.3%) and for combined HPV-18 E6 & E7 (0.2%) (Table 4-2). In HIV-positive controls, the overall antibody seroprevalence was 15.9% for HPV-16 L1, HPV-18 L1(15.7%), 0.5% for HPV-16 E6 and E7 and 0.2% for HPV-18 E6 and E7 (Table 4-3).

In general, we did not observe differences in seroprevalence to HPV types 16 and -18 L1 and (E6 and E7) antibodies by demographic and sexual/reproductive factors (p-value >0.05). Among HIV-negative controls, the prevalence of HPV-16 E7 antibody was higher among women that lived in

rural areas (8.6%) as compared to women who lived in urban areas (3.7%) (p-value for heterogeneity <0.005) (Table 4-2). A similar pattern was observed for HPV-18 E7 (rural (7.9%) vs urban (2.0%); p-value=0.001). Among HIV-positive controls, the prevalence of HPV-16 E6 & E7 antibodies decreased with an increase in the number of births up to 2 (p-value=0.006) (Table 4-3).

Table 4-2:Seroprevalence of antibodies to HPV-16 and -18 L1, E6 and E7 proteins in infection-unrelated cancer controls in HIV-negative women

		Serology markers							
	Total			HPV-16			HPV-18		
Characteristics	N=1865	HPV-16 L1 (%)	HPV-18 L1 (%)	E6 (%)	E7 (%)	E6 & E7 (%)	E6 (%)	E7 (%)	E6 & E7 (%)
Overall seroprevalence		14.3	16.3	2.9	4.0	0.5	2.4	2.5	0.2
Demographic									
Age									
25-34	206	25 (12.1)	34 (16.5)	2 (1.0)	5 (2.4)	1 (0.5)	6 (2.9)	3 (1.5)	0 (0.0)
35-44	638	86 (13.5)	93 (14.6)	19 (3.0)	21 (3.3)	4 (0.7)	17 (2.7)	12 (1.9)	1 (0.2)
45-54	1021	156 (15.3)	177 (17.3)	33 (3.2)	49 (4.8)	3 (0.3)	21 (2.1)	32 (3.1)	3 (0.3)
Chi-square trend (p-value)		0.166	0.352	0.133	0.053	0.472	0.345	0.067	0.358
Place of residence									
Rural	139	18 (13.0)	20 (14.4)	9 (6.5)	12 (8.6)	2 (1.6)	3 (2.2)	11(7.9)	1 (0.8)
Urban	1772	248 (14.4)	284 (16.5)	44 (2.6)	63 (3.7)	6 (0.4)	41 (2.4)	35 (2.0)	3 (0.2)
Chi-square heterogeneity age-adjusted (p-value)		0.406	0.512	0.202	0.005	0.042	0.886	<0.001	0.184
Period of interview									
1995-1999	115	22 (19.1)	26 (22.6)	5 (4.4)	7 (6.1)	1 (1.0)	4 (3.5)	4 (3.5)	0 (0.0)
2000-2004	373	61 (16.4)	65 (17.4)	15 (4.0)	17 (4.6)	4 (1.2)	5 (1.3)	7 (1.9)	2 (0.6)
2005-2009	663	86 (13.0)	95 (14.3)	19 (2.9)	27 (4.1)	1 (0.2)	15 (2.3)	14 (2.1)	1 (0.2)
2010-2016	714	98 (13.7)	118 (16.5)	15 (2.1)	24 (3.4)	2 (0.3)	20 (2.8)	22 (3.1)	1 (0.16)
Chi-square heterogeneity age-adjusted (p-value)		0.1757	0.123	0.202	0.455	0.142	0.368	0.538	0.454
Marital Status									
Never Married	422	56 (13.3)	67 (15.9)	11 (2.6)	12 (2.4)	2 (0.5)	5 (1.2)	11 (2.6)	0 (0.0)
Married	1002	134 (13.4)	161 (16.1)	30 (3.0)	47 (4.7)	5 (0.5)	34 (3.4)	23 (2.3)	4 (0.4)
Ever married	437	75 (17.2)	75 (17.2)	13 (3.0)	16 (3.7)	1 (0.2)	5 (1.1)	13 (3.0)	0 (0.0)

Characteristics	Total	Serology markers							
				HPV-16			HPV-18		
	N=1865	HPV-16 L1 (%)	HPV-18 L1 (%)	E6 (%)	E7 (%)	E6 & E7 (%)	E6 (%)	E7 (%)	E6 & E7 (%)
Chi-square heterogeneity age-adjusted (p-value)		0.227	0.926	0.976	0.278	0.814	0.007	0.798	0.180
Education Level									
None	123	22 (17.9)	21(17.1)	6 (4.9)	2 (1.6)	0 (0.0)	4 (3.3)	5 (4.1)	0 (0.0)
Primary	411	54 (13.1)	61 (14.8)	9 (2.2)	20 (4.9)	1 (0.3)	10 (2.4)	8 (2.0)	1 (0.3)
Secondary and above	1329	191 (14.4)	222 (16.7)	38 (2.9)	52 (3.9)	7 (0.6)	30 (2.3)	34 (2.6)	3 (0.2)
Chi-square adjusted for age trend (p-value)		**0.406**	**0.543**	**0.298**	**0.276**	**0.630**	**0.654**	**0.400**	**0.838**
Sexual/ reproductive history									
Parity									
0	34	4 (11.8)	5 (14.7)	2 (5.9)	1 (2.9)	1 (3.0)	0 (0.0)	1 (2.9)	0 (0.0)
1	316	30 (9.5)	44 (13.9)	10 (3.2)	15 (4.8)	4 (1.3)	11 (3.5)	9 (2.9)	1 (0.3)
2	485	68 (14.0)	77 (15.9)	18 (3.7)	15 (3.1)	1 (0.2)	13 (2.7)	11(2.3)	0 (0.0)
3	449	81 (18.0)	70 (15.6)	14 (3.1)	18 (4.0)	2 (0.5)	6 (1.3)	11 (2.6)	0 (0.0)
4+	486	67 (13.8)	88 (18.1)	9 (1.9)	23 (4.7)	0 (0.0)	12 (2.5)	14 (2.9)	3 (0.6)
Chi-square adjusted for age trend (p-value)		0.143	0.181	0.031	0.931	0.006	0.610	0.738	0.356
Number of sexual partners									
0-1	170	22 (12.9)	25 (14.7)	6 (3.5)	6 (3.5)	0 (0.0)	1 (0.6)	2 (1.2)	0 (0.0)
2-5	1116	161 (14.4)	182 (16.3)	36 (3.2)	53 (4.8)	7 (0.7)	28 (2.5)	31(2.8)	3 (0.3)
6+	204	32 (15.7)	32 (15.7)	5 (2.6)	6 (2.9)	1 (0.5)	9 (4.4)	5 (2.5)	0 (0.0)
Chi-square adjusted for age trend (p-value)		0.908	0.522	0.133	0.132	0.339	0.796	0.850	0.862
Unknown	375	52 (13.9)	65 (17.3)	7 (1.9)	10 (2.7)	0 (0.0)	6 (1.6)	9 (2.4)	1 (0.3)

Table 4-3:Seroprevalence of antibodies to HPV-16 and -18 L1, E6 and E7 proteins in infection-unrelated cancer controls in HIV-positive women

Characteristics	Total	Serology markers							
				HPV-16			HPV-18		
	N=667	HPV-16 L1 (%)	HPV-18 L1 (%)	E6 (%)	E7 (%)	E6 & E7 (%)	E6 (%)	E7 (%)	E6 & E7 (%)
Overall seroprevalence		15.9	15.7	2.4	3.3	0.5	1.5	3.2	0.2
Demographic									
Age									
25-34	136	24 (17.7)	15 (11.0)	5 (3.7)	5 (3.7)	1 (0.8)	1 (0.7)	1 (0.7)	0 (0.0)
35-44	300	42 (14.0)	50 (16.7)	7 (2.3)	12 (4.0)	1 (0.4)	5 (1.7)	10 (3.3)	0 (0.0)
45-54	231	40 (17.3)	40 (17.3)	4 (1.7)	5 (2.2)	1 (0.5)	4 (1.7)	10 (4.3)	1 (0.5)
Chi-square trend (p-value)		0.896	0.137	0.254	0.191	0.719	0.490	0.067	0.236
Place of residence									
Rural	28	2 (7.1)	4 (14.3)	0 (0.0)	1 (3.6)	0 (0.0)	1 (3.6)	1 (3.6)	0 (0.0)
Urban	639	104 (16.3)	101 (15.8)	16 (2.5)	21 (3.3)	3 (0.5)	9 (1.4)	20 (3.1)	1 (0.2)
Chi-square heterogeneity age-adjusted (p-value)		0.218	0.797	0.399	0.982	0.724	0.376	0.912	0.856
Period of interview									
1995-1999	7	1 (14.3)	1 (14.3)	1(14.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
2000-2004	90	9 (10.0)	11 (12.2)	4 (4.4)	4 (4.4)	1 (1.2)	3 (3.3)	1 (1.1)	1 (1.1)
2005-2009	230	32 (13.9)	36 (15.7	5 (2.2)	7 (3.0)	0 (0.0)	4 (1.7)	7 (3.0)	0 (0.0)
2010-2016	340	64 (18.8)	57 (16.8)	6 (1.8)	11 (3.2)	2 (0.6)	3 (0.8)	13 (3.8)	0 (0.0)
Chi-square heterogeneity age-adjusted (p-value)		0.138	0.872	0.109	0.898	0.572	0.287	0.701	0.036
Marital Status									
Never Married	231	38 (16.5)	36 (15.6)	4 (1.7)	4 (1.7)	0 (0.0)	1 (0.4)	5 (2.2)	0 (0.0)
Married	257	32 (12.5)	35 (13.6)	8 (3.1)	12 (4.7)	1 (0.4)	4 (1.6)	8 (3.1)	0 (0.0)
Ever married	179	36 (20.1)	34 (19.0)	4 (2.2)	6 (3.4)	2 (1.2)	5 (2.8)	8 (4.5)	1 (0.6)

Characteristics	Total	Serology markers							
				HPV-16			HPV-18		
	N=667	HPV-16 L1 (%)	HPV-18 L1 (%)	E6 (%)	E7 (%)	E6 & E7 (%)	E6 (%)	E7 (%)	E6 & E7 (%)
Chi-square heterogeneity age-adjusted (p-value)		0.101	0.448	0.573	0.171	0.178	0.188	0.673	0.488
Education Level									
None	24	2 (7.4)	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Primary	134	21 (15.7)	23 (17.2)	2 (1.5)	3 (2.2)	0 (0.0)	4 (3.0)	4 (3.0)	0 (0.0)
Secondary and above	505	83 (16.4)	81 (16.0)	13 (2.6)	19 (3.8)	3 (0.6)	6 (1.2)	17 (3.4)	1 (0.2)
Chi-square adjusted for age trend (p-value)		0.380	0.169	0.731	0.543	0.611	0.339	0.416	0.701
Sexual/ reproductive history									
Parity									
0	22	9 (40.9)	5 (22.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	142	20 (14.1)	15 (10.6)	3 (2.1)	7 (4.9)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
2	188	38 (20.2)	30 (16.0)	6 (3.2)	8 (4.3)	2 (1.1)	3 (1.6)	8 (4.3)	1 (0.6)
3	148	17 (11.5)	27 (18.2)	3 (2.0)	5 (3.4)	1 (0.7)	4 (2.7)	7 (4.7)	0 (0.0)
4+	131	17 (13.0)	21 (16.0)	3 (2.3)	2 (1.5)	0 (0.0)	2 (1.5)	4 (3.1)	0 (0.0)
Chi-square adjusted for age trend (p-value)		0.022	0.688	0.614	0.340	0.932	0.294	0.347	0.373
Number of sexual partners									
0-1	21	3 (14.3)	4 (19.1)	1 (4.8)	2 (9.5)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
2-5	355	55 (15.5)	52 (14.7)	5 (1.4)	9 (2.5)	0 (0.0)	8 (2.3)	8 (2.3)	1 (0.3)
6+	109	20 (18.4)	19 (17.4)	5 (4.6)	3 (2.8)	1 (1.0)	1 (0.9)	3 (2.8)	0 (0.0)
Chi-square adjusted for age trend (p-value)		0.869	0.875	0.260	0.518	0.041	0.143	0.291	0.602
Unknown	182	28 (15.4)	30 (16.5)	5 (2.8)	8 (4.4)	2 (1.2)	1 (0.6)	9 (5.0)	0 (0.0)

Being seropositive to HPV-16 antibodies was significantly associated with cervical cancer: combined HPV-16 E6 and 7 (AOR=69.20, 95% CI: 37.07-129.18), HPV-16 E6 (AOR=21.96, 95% CI:16.61-29.02), HPV-16 E7 (AOR =7.93, 95% CI 6.16-10.22) and HPV-16 L1 (AOR=1.74, 95% CI: 1.45-2.07). For HPV-18, antibody seroprevalence ORs for cervical cancer ranged from AOR =38.61 (95% CI: 15.13-98.53) for combined HPV-18 E6 & E7, AOR=8.94 (95% CI: 6.64-12.03) for HPV-18 E7, AOR =4.67 (95% CI: 3.30-6.50) for HPV-18 E6 and AOR=1.31 (95% CI:1.10-1.56) for HPV-18 L1 (Table 4-4).

Table 4-4: Association between HPV-16 and -18 L1 and (E6 and E7) seropositivity and cervical cancer

	Cases (N=1,346)	Controls (N=2,532)	
Serology markers	n (%) seropositive	n (%) seropositive	Adjusted OR (95% CI)
HPV-16 L1	332 (24.4)	373 (14.7)	1.74 (1.45-2.07)
HPV-18 L1	292 (21.7)	409(16.2)	1.31 (1.10-1.56)
HPV 16			
HPV-16 E6	467 (35.9)	70 (2.8)	21.96 (16.61-29.02)
HPV-16 E7	309 (24.7)	97 (3.8)	7.93 (6.16-10.22)
HPV-16 E6 & E7	209 (23.3)	11 (0.5)	69.20 (37.07-129.18)
HPV 18			
HPV-18 E6	120 (8.9)	54 (2.1)	4.67 (3.30-6.59)
HPV-18 E7	235 (18.6)	68 (2.7)	8.94 (6.64-12.03)
HPV-18 E6 &E7	64 (5.9)	5 (0.2)	38.61 (15.13-98.53)
HPV 16 or 18			
HPV-16/18 E6/E7	746 (56.8)	255 (10.1)	13.63 (11.32-16.41)

OR adjusted for age, HIV antibodies, education, number of sexual partners, place of residence, marital status and period of interview

In general, HPV L1 cervical cancer ORs were higher in HIV-positive women but lower in HIV-negative women. The AORs of cervical cancer for combined HPV-16 E6 & E7 seropositivity were 97.40 (95% CI: 46.68-203.23) in HIV-negative and 33.10 (95% CI:10.22-107.20) in HIV-positive women (Figure 4-3).

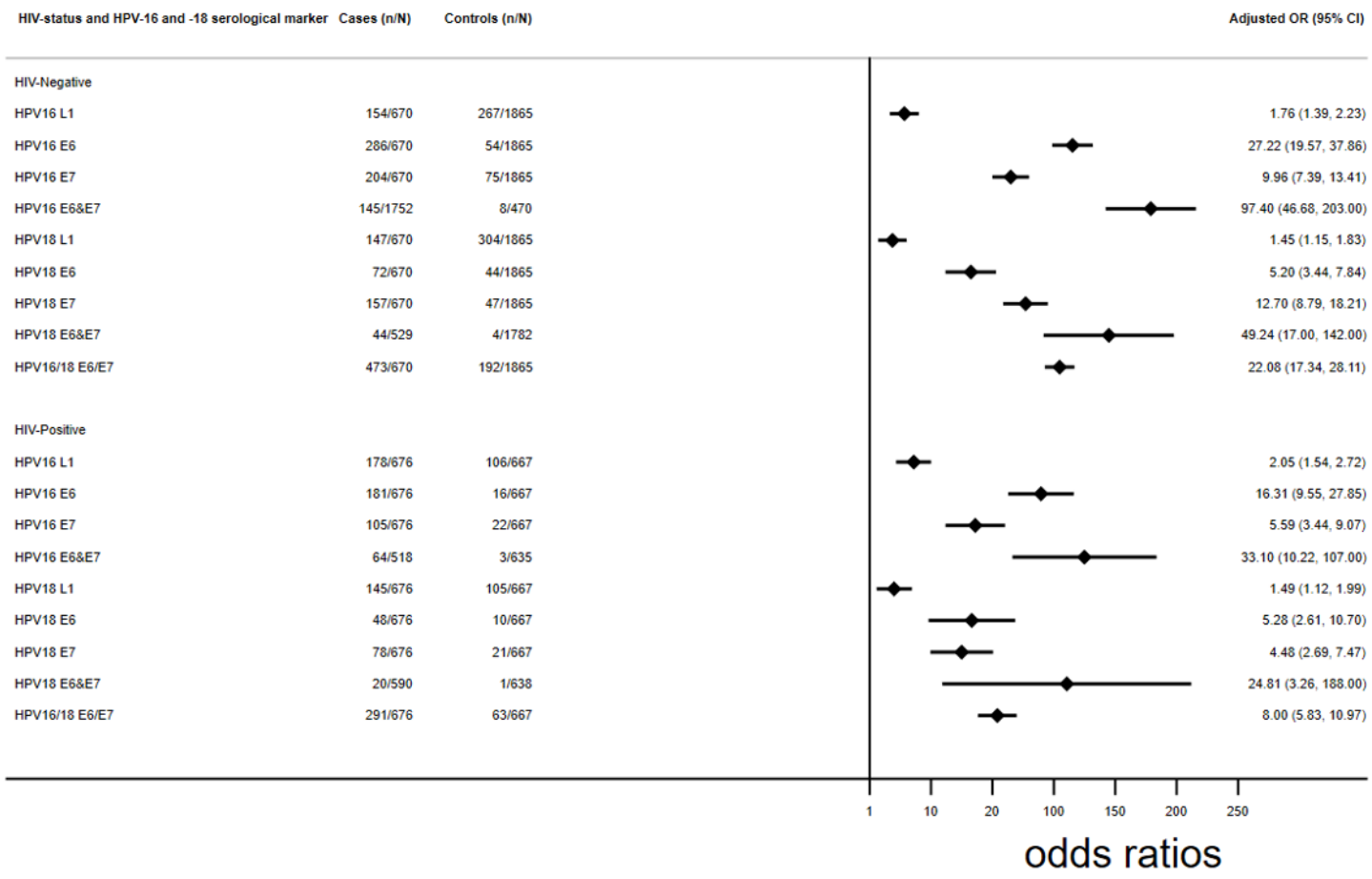


Figure 4-3: Relationship between HPV-16 and -18 (L1, E6 and E7) seropositivity and cervical cancer risk by HIV status. Odds ratios were adjusted for age, education level, number of sexual partners, marital status, place of residence and period of the interview.

Among HIV-positive women, HPV-16/18 E6/E7 antibodies had a sensitivity of 43.0%, specificity of 90.6% and AUC of 67% to detect cervical cancer. Among HIV-negative women, the sensitivity, specificity and AUC for HPV-16/18 E6/E7 antibodies were 70.6%, 89.7% and 80%, respectively, for the detection of cervical cancer. The sensitivity, specificity and AUC for discriminating cervical cancer based on HPV-16 E6 positivity were 26.8%, 97.2% and 62%, respectively, in HIV-positive women. For HIV-negative women who were HPV E6 seropositive, the sensitivity was 42.7%, specificity was 95.6% and the AUC 69% (Table 4-5).

Table 4-5: Clinical performance of HPV-16 and -18 antibody positivity as diagnostic markers for cervical cancer by HIV-status

Serology markers	HIV-positive			HIV-negative		
	Sensitivity (95% CI)	Specificity (95% CI)	AUC	Sensitivity (95% CI)	Specificity (95% CI)	AUC
HPV-16 E6	26.8 (23.5-30.3)	97.6 (96.1-98.6)	62	42.7 (38.9-46.5)	95.6 (94.5-96.4)	69
HPV-16 E7	15.5 (12.9-18.5)	96.7 (95.0-97.9)	56	30.4 (27.0-34.1)	96.0 (95.0-96.8)	63
HPV-16 E6 & E7	12.4 (9.6-15.5)	99.5 (98.6-99.9)	56	30.9 (26.7-35.2)	99.5(99.1-99.8)	65
HPV-18 E6	7.1 (5.3-9.3)	98.5 (97.3-99.3)	53	10.7 (8.5-13.3)	97.6 (96.8-98.3)	54
HPV-18 E7	11.5 (9.2-14.2)	97.9 (95.2-98.0)	54	23.4 (20.3-26.8)	97.1 (96.4-97.7)	60
HPV-18 E6 & E7	3.4 (2.1-5.2)	99.8 (99.1-100)	52	8.3 (6.1-11.0)	99.9 (99.5-99.9)	54
HPV-16/18 E6/E7	43.0 (39.3-46.9)	90.6 (88.1-92.7)	67	70.6 (67.0-74.0)	89.7(88.2-91.0)	80

4.4. Discussion

In this study, we assessed antibody seropositivity to each HPV-16 and -18 oncoprotein (E6 and E7) and L1 protein and calculated their association with cervical cancer. While the seroprevalence of HPV-16 L1 in the previous JCS study among HIV-seronegative women was higher [35] than in this study, ORs between HPV-16 L1 and cervical cancer were about the same (overall crude OR 2.2; 95% CI:1.8-2.6, vs 2.1; 1.5-2.7, Figure 4-3). Previous data on HPV-16 L1 seroprevalence in controls reflect different assay methods and cut-offs used and perhaps some cross-reactivity with other HPV types. Our key findings, using a larger sample size and more recent serological methods measuring a range of hrHPV types, showed the important role of HPV-16 and -18 (E6 and E7) oncoproteins in cervical cancer risk in both HIV-positive and HIV-negative cancer patients. Findings from our study confirmed that, in HIV-negative controls, HPV-16 E7 and HPV-18 E7 seroprevalence differed by place of residence. Among HIV-positive women, HPV-16 E6 & E7 seroprevalence increased with an increase in the number of births.

The higher seroprevalence of HPV-16 E6, HPV-16 E7 and HPV-18 E7 in women from rural areas might indicate a greater number of women with early undetected cervical or related cancer lesions. In rural South Africa, women have poor access to health care services. They are less likely to present themselves to cervical cancer screening services. In addition, they have less access to cancer diagnostic facilities, mainly found in tertiary hospitals in urban areas [172].

The high seroprevalence of HPV-16 E6 (35.9%) antibodies in cervical cancer cases found in our study are similar to the seroprevalence of HPV-16 E6 (32%) in cervical cancer cases reported in

the United Kingdom (35%) and India and Algeria (32%) [91,160]. We found a relatively low seroprevalence of HPV-16 E6 & E7 and HPV-18 E6 &E7 antibodies among controls in keeping with a low prevalence of HPV oncoprotein antibodies among women without cervical cancer [173]. Our study demonstrates that the high seroprevalence of HPV-16 E6 and E7 antibodies in cervical cancer development is in accordance with other studies from Russia and Italy [31,174].

Antibodies to the E6 and E7 oncoproteins are late markers of invasive cervical cancer [59]. In our study, seropositivity to the HPV-16 and -18 (E6 and E7) antibodies was associated with high risks of cervical cancer. Notably, seropositivity to HPV-16 (E6 & E7) and HPV-18 (E6 & E7) exhibited the highest ORs for cervical cancer (Figure 4-3). Similar findings were reported in a case-control study of patients recruited from India and Algeria [91]. Our findings support those from other cross-sectional, case-control and prospective studies, albeit each using different assays [22,23,59,175].

Individuals with suppressed immune systems are susceptible to chronic infection, including HPV [176]. Thus, HIV infection increases the risk of HPV persistence [177]. Existing evidence of the association between HPV (E6 and E7) and L1 proteins antibodies and HIV has come from studies on men who have sex with men [91,92,178]. In contrast, most existing studies on the association between antibodies to HPV-16 and -18 L1, E6 and E7 proteins and cervical cancer risk have been conducted in low HIV prevalence settings [31,59,161,165]. In our study, access to HIV data made it possible to stratify our analysis by HIV status. Our finding showed that antibody seropositivity to each of HPV-16 and -18 (E6 &E7) oncoproteins was strongly associated with the risk of cervical

cancer among HIV-positive women. Our data support the hypothesis that HIV plays an important role in the persistence of HPV infection.

Unexpected findings in our study were that HPV-16 and -18 (E6 and E7) oncoproteins seropositivity and cervical cancer risk were higher in HIV-negative cancer patients compared to HIV-positive cancer patients. Similar findings have been observed in previous studies of HPV antibodies among HIV-positive and HIV-negative patients [81,179–181]. In our study, the possible explanation could be that cervical cancer risk in HIV-positive women may be related to more than the two main types, HPV-16 and HPV-18, that we tested for, so their relative contribution appears attenuated (Additional Table 4-3). Other studies on HPV genotype distribution by HIV status in South Africa reported that HPV types 35, 58 and 33 were more commonly identified [182,183]. However, if this is indeed true, then the current HPV vaccine (16/18) may be less effective for HIV-positive women. Another possible explanation is that in HIV-positive patients, antibodies could be an indication of reactivation of a latent HPV.
In contrast, in HIV-negative patients, antibodies could be reinfection with a new type [81]. Another unexpected finding in our study was that the number of sexual partners was not associated with HPV L1 and (E6 and E7) antibodies. The plausible explanation could be that peak acquisition of HPV occurs at an age earlier than the age at the interview date in our study [184].

In the new guidelines for screening cervical cancer, the WHO has included HPV antibodies and oncoproteins as a potential future screening test [60]. We, therefore, assessed the clinical performance of HPV E6 /E7 antibodies as possible screening tests. We found that the sensitivity of the HPV antibodies for detecting cervical cancer was low, but the specificity was high among HIV-positive women. In contrast, sensitivity and specificity for detecting cervical cancer were high

among HIV-negative women. Predictive values would thus vary considerably depending on the background prior likelihood of disease. Our finding is in line with the previous findings on the sensitivity and specificity of HPV-16/18 E6/E7 antibodies as a screening test [22,136].

There are strengths to our study. The seroprevalence of HPV antibodies was conducted on a large sample size, and we used a high throughput multiplex serology, so all tests were done under the same laboratory conditions. The laboratory tests were conducted 'blind' without prior knowledge of the case/control status of the participants.

Nonetheless, our study has some limitations. Serology data on exposure and cervical cancer diagnoses were collected simultaneously. We did not have tumour HPV Deoxyribonucleic Acid (DNA) data to determine the causative type(s). Since only 50-70% of women with detectable HPV DNA in the cervix seroconvert [185], our study underestimates the true prevalence of HPV infection. The JCS did not recruit women with cervical pre-cancerous lesions.

Further, no cervical cancer staging information was available in the JCS. If E6/E7 serology is correlated to more advanced cancer, our results may overestimate the sensitivity of detecting early or pre-cancer lesions. Study results are based on Black women aged 25- 54 years recruited from the catchment area of Charlotte Maxeke hospital in Johannesburg and its major satellite hospitals and clinics. Therefore, our findings might not be generalizable to other South African regions and women aged 55 years and above.

Conclusions

Our data contribute to the evidence on the importance of HPV-16 and -18 E6 and E7 oncoproteins antibodies to discriminate cervical cancer from controls in a Black South African population. HPV L1 antibodies are considered exposure markers of past HPV infection, and HPV E6 and E7 are late markers of invasive cervical cancer. Furthermore, HPV-16 and -18 E6 and E7 seropositivity antibodies are strongly associated with cervical cancer in HIV-positive and HIV-negative patients.

Acknowledgements

We acknowledge the contributions of all authors in the ERICA-SA collaborative for their efforts in providing feedback to improve the final version of the manuscript.

Additional Tables and Figures

Additional Table 4-1:List of minor cancer types used as controls

Minor- Cancer type	**ICD-10-code**	**Frequency** **N= 162**
Bone	C40-41	22
Brain	C71	9
Fallopian tube	C57.0	3
Kidney	C64	12
Larynx	C32	12
Melanoma	C43	21
Meninges	C70	1
Peritemeum &retroperitonium	C48	5
Placenta	C58.9	32
Small intestine	C17	7
Soft tissue sarcoma	C49	18
Thymus	C37	5
Thyroid	C73	15

Additional Table 4-2:Comparison of HPV-16 and -18 (E6 and E7, L1) antibodies in cervical cancer Cases and Controls among young and older women

	Younger adult women (25-34 years)			Older adult women (35-54 years)		
	Cases (N=186)	Controls (N=342)		Cases (N=1160)	Controls (N=2,190)	
Serology markers	n (%) seropositive	n (%) seropositive	Odds Ratios (95%CI)	n (%) seropositive	n (%) seropositive	Odds Ratios (95%CI)
HPV-16 L1	52 (28.0)	49 (14.3)	1.89 (1.17-3.04)	280 (24.1)	324 (14.8)	1.66 (1.37-2.01)
HPV-16 E6	62 (33.3)	7 (2.1)	28.15 (11.77-67.32)	405 (34.9)	63 (2.9)	20.90 (15.60-28.00)
HPV-16 E7	36 (19.4)	10 (2.9)	9.18 (4.13-20.42)	273 (23.5)	87 (4.0)	7.39 (5.68-9.62)
HPV-16 E6 & E7	22 (16.7)	2 (0.6)	40.13 (8.59-187.48)	187 (21.9)	9 (0.4)	72.41 (36.57-143)
HPV-18 L1	45 (24.2)	49 (14.3)	1.51 (0.92-2.47)	247 (21.3)	360 (16.4)	1.24 (1.03-1.51)
HPV-18 E6	16 (8.6)	7 (2.1)	4.71 (1.80-12.34)	104 (9.0)	47 (2.2)	4.69 (3.25-6.77)
HPV-18 E7	25 (13.4)	4 (1.2)	17.15 (5.43-54.17)	210 (18.1)	64 (2.9)	8.36 (6.16-11.34)
HPV-18 E6 & E7	8 (5.0)	0 (0.0)	N/A	56 (5.9)	5 (0.2)	33.70 (13.17-86)
HPV-16 & 18 E6	13 (9.7)	0 (0.0)	N/A	36 (5.0)	5 (0.2)	25.61 (9.78-67)
HPV-16 & 18 E7	2 (1.6)	0 (0.0)	N/A	32 (4.3)	8 (0.4)	11.59 (5.14-26.13)
HPV-16/18 E6/E7	93 (50.0)	26 (7.6)	13.11 (7.59-22.67)	67 1(57.8)	229(10.5)	13.34 (10.98-16.21)

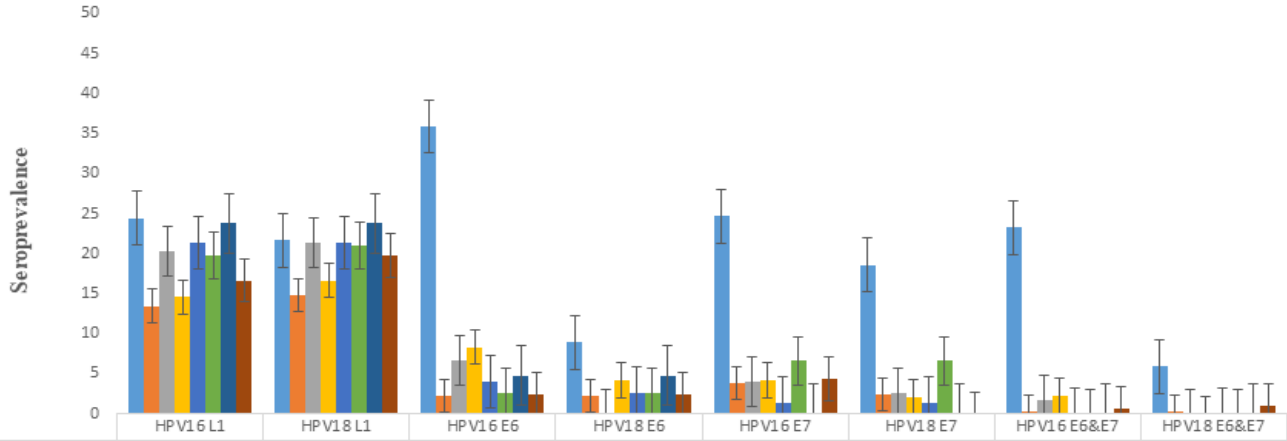
Odds ratios adjusted for HIV antibodies, education level, number of sexual partners, marital status, place of residence and period of interview

Additional Table 4-4-1: Seroprevalence of antibodies against HPV-16 and -18 (L1 E6 and E7) by HIV-Status

seropositive	HIV-positive n (%)	HIV-negative n (%)
HPV L1 (16&18)	169 (14.7%)	288 (12.9)
HPV E6 (16&18)	24 (2.1)	30 (1.4)
HPV E7 (16&18)	9 (0.8)	33 (1.6)

Additional Table 4-2: Clinical performance of HPV-16 and -18 antibodies as a diagnostic marker for cervical cancer

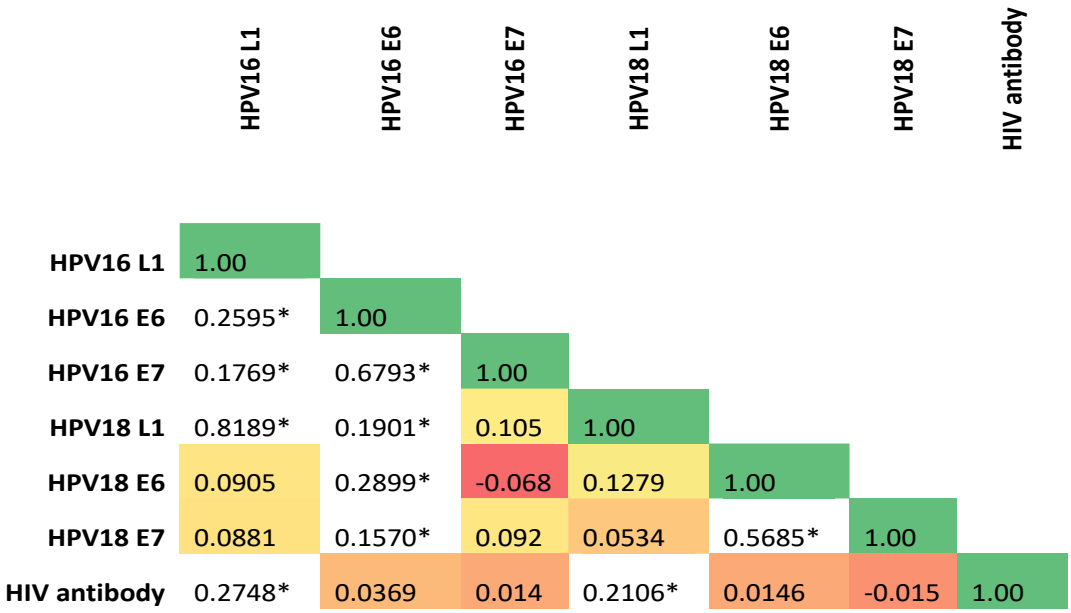
	Diagnostic		
Serology markers	Sensitivity (95% CI)	Specificity (95% CI)	AUC
HPV-16 E6	34.7 (32.2-37.3)	97.0 (96.4-97.6)	66
HPV-16 E7	23.0 (20.7-25.3)	96.2 (95.3-96.9)	60
HPV-16 E6 & E7	21.2 (18.6-23.8)	99.5 (99.2-99.8)	60
HPV-18 E6	8.9 (7.4-10.6)	97.8 (97.3-98.3)	53
HPV-18 E7	17.5 (15.5-19.6)	97.3 (96.6-97.9)	57
HPV-18 E6 & E7	5.7 (4.4-7.2)	99.8 (99.5-99.9)	53
HPV-16/18 E6/E7	56.8 (54.1-59.4)	89.3 (88.2-90.4)	73



	HPV16 L1	HPV18 L1	HPV16 E6	HPV18 E6	HPV16 E7	HPV18 E7	HPV16 E6&E7	HPV18 E6&E7
Cervical (53)	24.4	21.7	35.9	8.9	24.7	18.6	23.3	5.9
Breast(C50)	13.5	14.9	2.2	2.2	3.8	2.5	0.3	0.2
Colon(C18-20)	20.3	21.3	6.6	0.0	4.1	2.6	1.7	0.0
Oesophagus(C15)	14.6	16.7	8.3	4.2	4.2	2.1	2.3	0.0
Endometrium(C54-55)	21.3	21.3	4.0	2.7	1.3	1.3	0.0	0.0
Lung Cancer(C33-34)	19.7	21.1	2.6	2.6	6.6	6.6	0.0	0.0
Pancreas(C25)	23.8	23.8	4.8	4.8	0.0	0.0	0.0	0.0
minor cancer types	16.7	19.8	2.5	2.5	4.3	0.0	0.7	1.0

Chi-square (d.f. 6)	**12.57**	**11.32**	**0.71**	**6.39**	**3.69**	**6.94**	**11.48**	**2.89**
p-value	**0.054**	**0.079**	**0.870**	**0.381**	**0.719**	**0.326**	**0.075**	**0.823**

Additional Figure 4-1: Age-adjusted seroprevalence of HPV-related antibody markers in cervical cancer cases and other infection-unrelated cancer controls and p-value for heterogeneity among the infection-unrelated cancer controls (i.e. breast, colon, oesophagus, endometrium, lung, pancreas, other minor types). The seropositivity of the age-adjusted HPV L1 and (E6 and E7) antibodies in each of the infection-unrelated cancer patients (controls) were similar (p-heterogeneity >0.05).



Additional Figure 4-2: Correlation between HPV-16 and -18 proteins L1, E6 and E7 antibodies and HIV antibodies, R-values > 0.8 shows high correlation. (* Significance at 0.05). The colour indicates the intensity of the correlation. Green indicates positive values; red indicates negative ones; orange shows R-values greater than 0.05, and dark orange indicates R-values < 0.05 but not statistically significant.

5.0. Chapter Five: Ranking lifestyle risk factors for cervical cancer among Black women: A Case-Control study from Johannesburg, South Africa

Authors: Mwiza Gideon Singini, Freddy Sitas, Debbie Bradshaw, Wenlong Carl Chen, Melitah Motlhale, Abram Bunya Kamiza, Chantal Babb de Villiers, Cathryn M. Lewis, Mazvita Muchengeti, Tim Waterboer, Christopher G. Mathew, Robert Newton and Elvira Singh

Published in (PLoS ONE 16(12): e0260319) 2021

5.1. Introduction

This chapter presents the last part of the study by investigating the role of other known co-factors for cervical cancer. In addition, the chapter elucidates the importance of cofactors in developing cervical cancer. It follows from chapters three and four, where infection with hrHPV types demonstrates that it does not always result in the progression of invasive cervical cancer. This chapter, therefore, strongly suggests the involvement of other cofactors in the carcinogenesis of cervical cancer. In addition, it ranks them in order of importance in an urban setting. The rank order of risks could be used for prevention strategies of cervical cancer. In this chapter, we also provide the methodology for selecting appropriate cancer controls based on different types of exposures. We published this work for this chapter in PloS One Journal in 2021.

Abstract

Background

Aside from HPV, the role of other risk factors in cervical cancer, such as age, education, parity, sexual partners, smoking, and HIV, has been described but never ranked in order of priority. We evaluated the contribution of several known lifestyle co-risk factors for cervical cancer among Black South African women.

Methods

We used participant data from the JCS, a case-control study of women recruited mainly at Charlotte Maxeke Johannesburg Academic Hospital between 1995 and 2016. A total of 3,450 women in the study had invasive cervical cancers, 95% of which were squamous cell carcinoma. Controls were 5,709 women with cancers unrelated to exposures of interest. Unconditional logistic regression models were used to calculate adjusted odds ratios (AOR) and 95% confidence intervals (CI). We ranked these risk factors by their population-attributable fractions (PAF), which take the local prevalence of exposure among the cases and risk into account.

Results

Cervical cancer in decreasing order of priority was associated with (1) being HIV positive (AOR=2.83, 95% CI:2.53-3.14, PAF=17.6%), (2) lower educational attainment (AOR =1.60, 95% CI: 1.44-1.77, PAF=16.2%), (3) higher parity (3+ children vs 2-1 children (AOR=1.25, 95% CI:1.07-1.46, PAF=12.6%), (4) hormonal contraceptive use (AOR=1.48, 95% CI: 1.24-1.77, PAF=8.9%), (5) heavy alcohol consumption (AOR =1.44 , 95% CI:1.15-1.81, PAF=5.6%), (6) current smoking (AOR=1.64, 95% CI:1.41-1.91,PAF=5.1%), and (7) rural residence (AOR =1.60, 95% CI:1.44-1.77, PAF=4.4%).

Conclusion

This rank order of risks could be used to target educational messaging and appropriate interventions for cervical cancer prevention in South African women.

Keywords: cervical cancer, HIV, smoking, alcohol consumption, South Africa

Background

Cervical cancer is the fourth most frequently occurring cancer type and the fourth leading cause of death from cancer among women globally [3]. In sub-Saharan Africa (SSA), more than 101,423 new cases and 76,444 cervical cancer deaths occur yearly [3]. In South Africa, the age-standardised incidence rate of cervical cancer, as reported by GLOBOCAN, is 44.4 per 100,000 women per year [3]. According to the 2017 South African National Cancer Registry report, there were 5,630 new histologically confirmed cases of cervical cancer amongst Black women with a lifetime risk (0-74 years) of 1 in 33 women [186].

The main risk factor for cervical cancer and its premalignant lesions is persistent infection with hrHPV [48]. A previous JCS showed a seroprevalence of 78% for -anti-HPV-16 antibodies in cervical cancer cases [35]. However, at least 90% of women with hrHPV infection do not develop cervical cancer [48]. This suggests that other environmental cofactors acting jointly with hrHPV elevate the risk of cervical carcinogenesis [187].

Epidemiological studies have indicated HIV, parity, smoking, alcohol consumption, contraceptive use, age, education and number of sexual partners to be cofactors of cervical cancer pathogenesis [101,187–192], but these cofactors vary in prevalence (and possibly the risk) in different settings. Understanding the prevalence levels of different cofactors and their risk for cervical cancer in a local setting may help identify risk profiles to target cervical cancer prevention.

In South Africa, studies on some lifestyle-related cofactors for cervical cancer were published about 10 years ago from the JCS [193–195]. These studies focused separately on individual cofactors such as HIV [195], smoking [194] or contraceptive use [193]. Among Black South African women, little is known about the relative importance of these cofactors for cervical cancer.

In South Africa, the JCS was established in 1995 to redress at that time the historical paucity of epidemiological cancer research among Black South Africans [33]. The JCS aimed to examine whether key known and emerging risk factors for cancer in women of mainly European ancestry applied to patients of African ancestry in Johannesburg, South Africa. These aims evolved into measuring the importance of known and emerging risk factors for cancer in a local setting.

We analysed JCS lifestyle data to evaluate the contribution of different cofactors in the pathogenesis of cervical cancer among Black South African women. In the current study, we used a subset of the JCS female samples and focused on ages 25 to 64 to align with current cervical screening guidelines. We also allowed for a more comparable assessment of the contribution of different cofactors in rank order to the risk of cervical cancer.

5.2. Methods

5.2.1. Setting and participants

The details of the JCS have been described elsewhere [33]. However, briefly, the JCS recruited over 26,000 Black South African patients (both sexes) who were newly diagnosed with incident cancer between 1995 and 2016. Recruitment took place mainly at Charlotte Maxeke-Johannesburg Academic Hospital medical oncology, radiotherapy, and associated peripheral clinics. Using a structured questionnaire, trained interviewers collected self-reported data on demographics and key lifestyle risk factors from consenting participants. They took peripheral blood samples for HIV and other analyses. The questionnaire included questions on the following: sociodemographic factors such as; place of birth and residence, marital status, education, and the home language of parents. Environmental exposures such as the method of cooking and heating. Lifestyle factors include smoking by type of tobacco and amounts smoked, snuff (sniffed tobacco) use, alcohol consumption by type, parity, use of oral and injectable contraceptives, and the number of sexual partners. On occupations, self-reported use of Anti-Retroviral Therapy (ART) (since 2005), PAP smear (2001) and self-reported history of diabetes. The JCS and the current study were approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number for the current study. is M200252). In the JCS, participants gave written informed or witnessed consent to a once-off interview and optional blood draw and to have their information and blood sample anonymized. Any future investigations require approval of the University of the Witwatersrand Human Research Ethics Committee HREC [33].

5.2.2. Selection of cases and controls

The JCS is amenable to a case-control design and analysis. Cases were women who were newly diagnosed with invasive cervical cancer (histologically confirmed squamous cell carcinoma and adenocarcinoma), and controls were designated as women with newly diagnosed cancers that have no known relationship with the exposures of interest [33].

A total of 26,263 participants were enrolled in the JCS between 1995 to 2016. We excluded 8,677 (33.0%) males, 3,105 (17.6%) women older than 65 years or younger than 25 years, 663 (4.6%) non-cancer participants, 945(6.9%) non-South Africans, 1 with missing data, 640 (5.0%) with missing HIV-status and 691 (5.7%) with primary site unknown malignancy. From those with cancer of the cervix, we excluded International Classification of Diseases for Oncology (ICD-O) codes such as (ICD-O morphology: 8010-8050 epithelial neoplasm (n=98, 2.6%), (ICD-O morphology: 8000 and 8001) not otherwise specified (n=9, 0.2%), (ICD-O morphology: 8560, 8570 and 8574) complex epithelial neoplasms (n=108, 2.8%) and other minor histological types (ICD-O morphology: 8098,8170, 8200, 8272, 8310, 8441,8460, 8480-8490, 8500,8650 and 8931-9100) (n=64, 1.7%) Figure 5-1.

Figure 5-1 also outlines the criteria for selecting cases and controls. The control groups were arranged into four sets. Each set comprised women diagnosed with different cancer types unrelated to the exposures of interest (infection, smoking, alcohol, parity, number of sexual partners and use of hormonal contraceptives) [33,196,197] Figure 5-1. Cancer controls unrelated to infection constituted the highest number of controls Table S 5-1. The demographic analyses excluded 2,009 (26.0%) women with infections-related cancers. Analyses relating to smoking excluded 2,218 (28.7%) women with cancers related to smoking and infections. Analyses relating to alcohol excluded 6,800 (60.4%) women with cancers related to alcohol and infections. Analyses relating to hormonal contraceptives excluded 6,458 (57.4%) women with cancers related to hormonal contraceptives and infections. Of the remaining 9,249 women, 3,540 (38.3%) had invasive cervical cancer (defined as cases), and 5,709 (61.7%) had other cancers (defined as controls) that were not related to the exposure of interest Figure 5-1.

5.2.3. Definition of exposures

For other demographic factors, women were grouped according to the period of the interview (1995-1999, 2000-2004, 2005-2009, 2010-2016), education level (none, primary, secondary and tertiary and unknown), marital status (never married, married, ever married and unknown) and place of residence (rural, urban and unknown).

Women's HIV status (positive and negative) was based on Abbott Axysm HIV1/2 gO Microparticle ELISA (1995 to 2005) and the Vironostika (HIV UniForm II plus O) micro ELISA (2006 to 2016) test results [195], while the other cofactors were self-reported. Use of hormonal contraceptives was categorized into (never (injectable and oral), ever oral/ not injectable, ever injectable/

not oral, ever oral, injectable and ever injectable and oral) and unknown [193]. Parity was categorized into 1-2, >=3 children and unknown. We excluded 168 (1.9%) women who had never given birth (on the assumption that some may have had pre-existing unreported gynaecological conditions or hysterectomies). The number of sexual partners for the participants was grouped into 0-1, 2-5, >=6 and unknown.

Women's smoking status was classified into never (non-smokers), ex-smoker, current smoker and unknown. The current smokers included those women who reported having quit smoking within 5 years of the interview (diagnosis) to reduce the likelihood of reverse causation [194]. We calculated total alcohol consumption based on different alcoholic beverages consumed: spirits (homebrew and commercial), beer (homebrew and commercial), wine, maize and sorghum, and ethanol content from each type of alcoholic beverage and the number of drinks per day. The average number of drinks per week was used to calculate the total alcohol consumption [198]. We defined one drink as having drunk 15.7 grams of unmixed alcohol beverage, equivalent to one bottle of beer of 340ml that contains 4% ethanol. Based on the Centre for Disease Control (CDC) definitions [199], we categorized women's self-reported consumption of alcoholic beverages into never, light (31.4 grams or less of alcohol per week) and heavy (47.1 grams or more of alcohol per week).

5.2.4. Statistical Analysis

Demographic characteristics were summarized using frequencies and percentages for the categorical variables, medians and the interquartile range (IQR) for the continuous variable that was not

normally distributed. We then analysed the relationship between the six cofactors with cervical cancer, using the appropriate set of cancer controls for each analysis Figure 5-1.

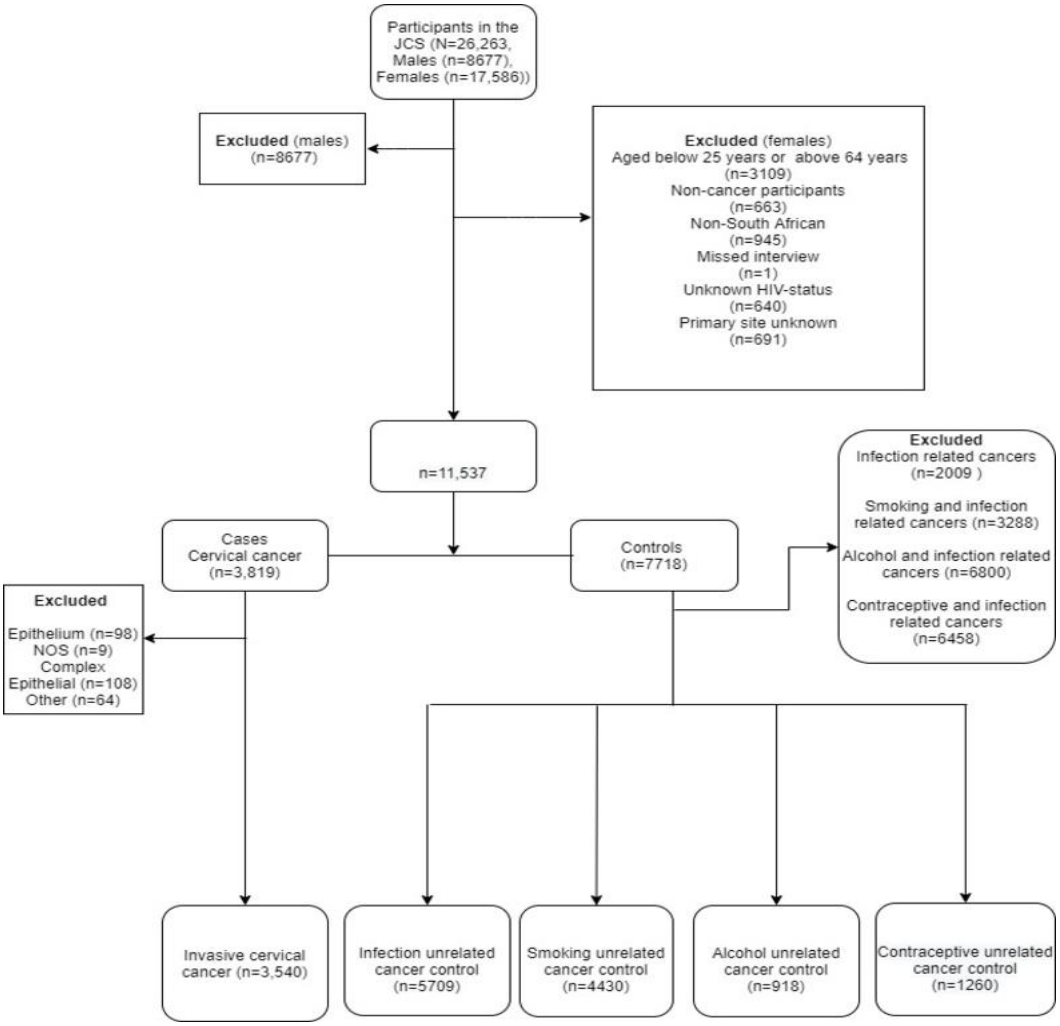


Figure 5-1: Data flow diagram on the selection exposure unrelated cancer controls.

We assessed for HIV status in model 1, parity in model 2, smoking in model 3, alcohol consumption in model 4, hormonal contraceptive use in model 5, and the number of sexual partners in model 6. We fitted our data using a backward stepwise multivariable unconditional logistic regression to estimate the unadjusted and adjusted odds ratios (AOR) and 95% confidence intervals (CI). In the final multivariable analysis of each model, we returned to the model variables where the

literature supported an association with cervical cancer. We ran 6 different models with different primary exposures. We adjusted simultaneously for the potential confounders: age (grouped), the interview period, marital status, place of residence and education level. We performed a test for heterogeneity on categorical variables and a score test for trends in the odds ratios on ordinal categorical variables using unconditional logistic regression Table 5-2. We used a Pearson correlation matrix to test for multicollinearity between independent variables in each model [200]. We used the Mantel-Haenszel Chi-square test to assess differences in the prevalence of cofactors across different cancer types in different sets of controls Table 5-3.

5.2.5. Population attributable fractions

We calculated population attributable fraction (PAF) using Miettinen's method given by PAF= $P_{ei}\left(\frac{OR_{adj}-1}{OR_{adj}}\right)$ [120]; where P_{ei} is the proportion of cases exposed to a particular cofactor, and i is the exposure level. When a variable had three categories, we added the PAF for the categories. A PAF % represents the proportion of the disease attributable to a particular exposure. We then ranked the cofactors which were significant based on their PAF. All the statistical tests were done using STATA software version 16.0 (Stata Corp, college station, Tx). Statistical significance was considered at a two-sided α-level of 0.05.

5.3. Results

Controls were relatively older compared to cases (Median age 50 years (IQR: 42-57) versus 48 years (IQR: 41-55)). The highest percentage of participants were in the age group of 45-54 years: 36.4% among cervical cancer cases and 33.8% among controls. Most of the study participants (cases = 84.2% and controls = 90.4%) lived in urban areas. More than half of the participants (58.5%) achieved a secondary or greater level of education Table 5-1.

Table 5-1: Demographics: Cervical cancer cases and infection unrelated cancer control participants from the JCS

Characteristics	Total (N=9249 (100%)) N (%)	Cases (N=3540 (100%)) n (%)	Controls (N=5709 (100%)) n (%)
Cervical cancer types			
SCC		3364 (95.0)	
Adenocarcinoma		176 (5.0)	
Demographics			
Age			
Median (IQR)	49 (42-56)	48 (41-55)	50 (42-57)
Age			
25-34	795 (8.6)	286 (8.1)	525 (8.9)
35-44	2410 (26.1)	1010 (28.5)	1400 (24.5)
45-54	3220 (34.8)	1288 (36.4)	1932 (33.8)
55-64	2824(30.5)	956 (27.1)	1868 (32.7)
Period of interview			
1995-1999	1619 (17.5)	766 (21.6)	853 (14.8)
2000-2004	1455 (15.7)	477 (13.5)	978 (17.1)
2005-2009	2544 (27.5)	994 (28.1)	1550 (27.2)
2010-2016	3631 (39.3)	1303 (36.8)	2328 (40.8)
Marital Status			
Never Married	2224 (24.1)	861 (24.3)	1363 (23.9)
Married	4152 (44.8)	1581 (44.7)	2571 (45.0)
Ever married	2848 (30.8)	1088 (30.7)	1760 (30.8)
Missing data	25 (0.3)	10 (0.3)	15 (0.3)
Place of Residence			
Rural	1069 (11.5)	546 (15.4)	524 (9.1)
Urban	8181 (88.1)	2982 (84.2)	5190 (90.4)
Missing data	39 (0.4)	13 (0.4)	26 (0.5)

Characteristics	Total (N=9249 (100%)) N (%)	Cases (N=3540 (100%)) n (%)	Controls (N=5709 (100%)) n (%)
Education Level			
None	1037 (11.2)	515 (14.6)	522 (9.1)
Primary	2772 (30.0)	1202 (34.0)	1570 (27.5)
Secondary	4930 (53.3)	1707 (48.2)	3223 (56.5)
Tertiary	479 (5.2)	97 (2.7)	379 (6.6)
Missing data	34 (0.4)	19 (0.5)	15 (0.3)

Cancer Controls are unrelated to infection, Ever married includes widowed and divorced, IQR=Interquartile range, SCC = Squamous Cell Carcinoma

Cases had nearly three times the risk of being HIV positive compared to controls (AOR 2.83, 95% CI: 2.54-3.15). After controlling for the common set of confounders, a strong trend of elevated risk with higher parity was observed relative to 1-2 children, with odds ratios of 1.25 (95% CI: 1.07-1.46) for women reporting three or more children. Table 5-2. Compared to never-smokers, the risk of cervical cancer significantly increased among women who were current smokers (AOR 1.55, 95% CI: 1.34-1.80) and ex-smokers (AOR 1.26, 95% CI: 1.02-1.55). The odds ratios for cervical cancer were 1.44 (95% CI: 1.26 -1.65) for women in the age group 35-44 years and 1.33 (95% CI: 1.18-1.50) for those in the age group 45-54 years old compared to women in the age group 55-64 years. The risk of cervical cancer increased among married women compared to never married (AOR 1.14, 95% CI: 1.01-1.28). The risk of cervical cancer among women who consumed at least 47.1 grams of alcohol per week (heavy alcohol consumption) was 44% higher (AOR 1.44, 95% CI: 1.15-1.81) compared to women who reported having never consumed alcohol. Cases were more likely than controls to have used injectable contraceptives (AOR 1.34, 95% CI: 1.11-1.61) and used oral or injectable contraceptives (AOR 1.17, 95% CI: 1.01-1.37). A lower level of educational attainment was associated with an increased risk of cervical cancer: Education (None versus Secondary and above AOR 2.01, 95% CI: 1.73-2.34) and (Primary versus Secondary and above OR_{ad} 1.60, 95% CI: 1.44-1.77). Living in rural compared to urban areas increased the risk of cervical cancer by 62% (AOR 1.62,95% CI: 1.44-1.77).

Table 5-2: Multivariable analysis of lifestyle risk factors and cervical cancer participants from the JCS

Characteristics	Total (N=9249) N (%)	Cases (N=3540) n (%)	Controls (N=5709) n (%)	Unadjusted OR (95%CI)	Adjusted Odds Ratios (95% CI) using appro-priately chosen controls	Case-control comparison p-value
Demographic factors						
Age						
25-34	795 (8.6)	286 (8.1)	525 (8.9)	1.10 (0.93-1.29)	1.00 (0.96-1.37)	0.906
35-44	2410 (26.1)	1010 (28.5)	1400 (24.5)	1.41 (1.26-1.58)	1.34 (1.17 -1.52)	<0.001
45-54	3220 (34.8)	1288 (36.4)	1932 (33.8)	1.30 (1.17-1.45)	1.28 (1.14-1.43)	<0.001
55-64	2824(30.5)	956 (27.1)	1868 (32.7)	1.00	1.00	
p-value (trend)				<0.001	0.016	
Period of interview						
1995-1999	1619 (17.5)	766 (21.6)	853 (14.8)	1.00	1.00	
2000-2004	1455 (15.7)	477 (13.5)	978 (17.1)	0.54 (0.47-0.63)	0.53 (0.45-0.62)	<0.001
2005-2009	2544 (27.5)	994 (28.1)	1550 (27.2)	0.71 (0.63-0.81)	0.67 (0.59-0.77)	<0.001
2010-2016	3631 (39.3)	1303 (36.8)	2328 (40.8)	0.62 (0.55-0.70)	0.59 (0.51-0.68)	<0.001
p-value (trend)				<0.001	<0.001	
Marital Status						
Never Married	2224 (24.1)	861 (24.3)	1363 (23.9)	1.00	1.00	
Married	4152 (44.8)	1581 (44.7)	2571 (45.0)	0.99 (0.87-1.12)	1.14 (1.01-1.28)	0.023
Ever married	2848 (30.8)	1088 (30.7)	1760 (30.8)	1.00 (0.87-1.14)	1.11 (0.98-1.27)	0.061
Missing data	25 (0.3)	10 (0.3)	15 (0.3)	-	-	
p-value (heterogeneity)				0.880	0.156	
Place of residence						
Rural	1069 (11.5)	546 (15.4)	524 (9.1)	1.85 (1.62-2.01)	1.62 (1.41-1.86)	<0.001
Urban	8181 (88.1)	2982 (84.2)	5190 (90.4)	1.00	1.00	
Missing data	39 (0.4)	13 (0.4)	26 (0.5)	-	-	

Characteristics	Total (N=9249) N (%)	Cases (N=3540) n (%)	Controls (N=5709) n (%)	Unadjusted OR (95%CI)	Adjusted Odds Ratios (95% CI) using appro-priately chosen controls	Case-control comparison p-value
p-value (heterogeneity)				<0.001	<0.001	
Education Level						
None	1037 (11.2)	515 (14.6)	522 (9.1)	1.97 (1.72-2.25)	2.01 (1.73-2.34)	<0.001
Primary	2772 (30.0)	1202 (34.0)	1570 (27.5)	1.53 (1.39-1.68)	1.60 (1.44-1.77)	<0.001
Secondary and above	5406 (58.5)	1804 (51.0)	3223 (63.1)	1.00	1.00	
Missing data	34 (0.4)	19 (0.5)	15 (0.3)			
p-value (heterogeneity)				<0.001	<0.001	
Lifestyle factors						
HIV Status					Model 1	
Negative	6759 (72.8)	2217 (62.6)	4542 (79.0)	1.00	1.00	
Positive	2550 (27.2)	1323 (37.4)	1207 (21.0)	2.32 (2.10-2.54)	2.83 (2.53-3.14)	<0.001
Missing data	-	-	-		-	
p-value (heterogeneity)				<0.001	<0.001	
Parity					Model 2	
1-2	1595 (33.2)	1138 (32.2)	457 (35.5)	1.00	1.00	
3+	3021 (668.9)	2288 (65.0)	733 (57.0)	1.49 (1.36-1.63)	1.25 (1.07-1.46)	0.005
Missing data	190 (4.0)	94 (2.7)	96 (7.5)	0.65 (0.50-0.83)	0.57 (0.44-0.74)	0.002
p-value (trend)				<0.001	0.002	
Smoking					Model 3	
Never	6737(83.3)	2830 (79.9)	3807 (85.9)	1.00	1.00	
Ex-Smoker	424 (5.3)	209 (5.9)	215 (4.8)	1.31 (1.07-1.59)	1.26 (1.02-1.55)	0.030
Current	901 (11.3)	498 (14.1)	403 (9.2)	1.66 (1.44-1.91)	1.55 (1.34-1.80)	<0.001
Missing data	8 (0.1)	3 (0.1)	5 (0.1)	-	-	
p-value (heterogeneity)				<0.001	<0.001	
Alcohol consumption					Model 4	
Never	3538 (79.3)	2781 (78.6)	757 (82.5)	1.00	1.00	

Characteristics	Total (N=9249) N (%)	Cases (N=3540) n (%)	Controls (N=5709) n (%)	Unadjusted OR (95%CI)	Adjusted Odds Ratios (95% CI) using appro-priately chosen controls	Case-control comparison p-value
Light	243 (5.5)	194 (5.5)	49 (5.3)	1.08 (0.77-1.49)	1.20 (0.86-1.68)	0.287
Heavy	677 (15.2)	565 (16.0)	112 (12.2)	1.37 (1.10-1.71)	1.44 (1.15-1.81)	0.002
Missing data	-	-	-	-	-	
p-value (heterogeneity)				0.017	0.078	
Contraceptive use					Model 5	
Never (injectable and oral)	1833 (38.2)	1262 (35.7)	571 (45.3)	1.00	1.00	
Ever Oral/ not injectable	578 (12.0)	408 (11.5)	170 (13.5)	1.09 (0.89-1.33)	1.09 (0.88-1.36)	0.847
Ever Injectable/ not oral	1526 (31.8)	1226 (34.5)	305 (24.2)	1.81 (1.54-2.13)	1.34 (1.11-1.61)	0.002
Ever oral and Injectable	844 (17.6)	637 (18.0)	207 (16.4)	1.39 (1.16-1.68)	1.22 (1.00-1.50)	0.366
Ever injectable and/ or oral	2948 (61.4)	2266 (64.0)	709 (54.5)	1.50 (1.32-1.71)	1.17 (1.01-1.37)	0.039
Missing data	19 (0.4)	12 (0.3)	7 (0.6)	-	-	
p-value (heterogeneity)				<0.001	0.183	
Number of sexual partners					Model 6	
0-1	661 (7.2)	233 (6.6)	428 (7.5)	1.00	1.00	
2-5	5384 (58.0)	2123 (60.0)	3243 (56.8)	1.20 (1.01-1.42)	1.15 (0.96-1.37)	0.129
6+	1007 (10.9)	397 (11.2)	610 (10.7)	1.20 (0.98-1.47)	1.11 (0.89-1.38)	0.298
Missing data	2215 (24.0)	787 (22.2)	1428 (25.0)	1.01 (0.84-1.21)	1.09 (0.88-1.34)	0.378
p-value (trend)				0.040	0.020	

Notes:

a. The total for alcohol, smoking and contraceptive does not add up to the total because some of the cancers were removed from the list of controls (see Table 3 in the appendix).
b. Odds Ratios were adjusted for age, education level, marital status, period of the interview, and place of residence (rural and urban).
c. Each model's sets of controls differ depending on the association between exposure and cervical cancer.

Our estimated PAF of cervical cancer for the 7 modifiable cofactors ranged from HIV(positive) (17.6%), lower education attainment (no education or primary) (16.9%), higher parity (3+ children) (12.6%), contraceptive use (Ever injectable and/ or oral) (8.9%), alcohol consumption (light and heavy) (5.6%), smoking (ex-smoker and current smoker) (5.1%) and 4.4% for the place of residence (rural) Figure 5-2. The number of sexual partners was excluded as it was not statistically significant.

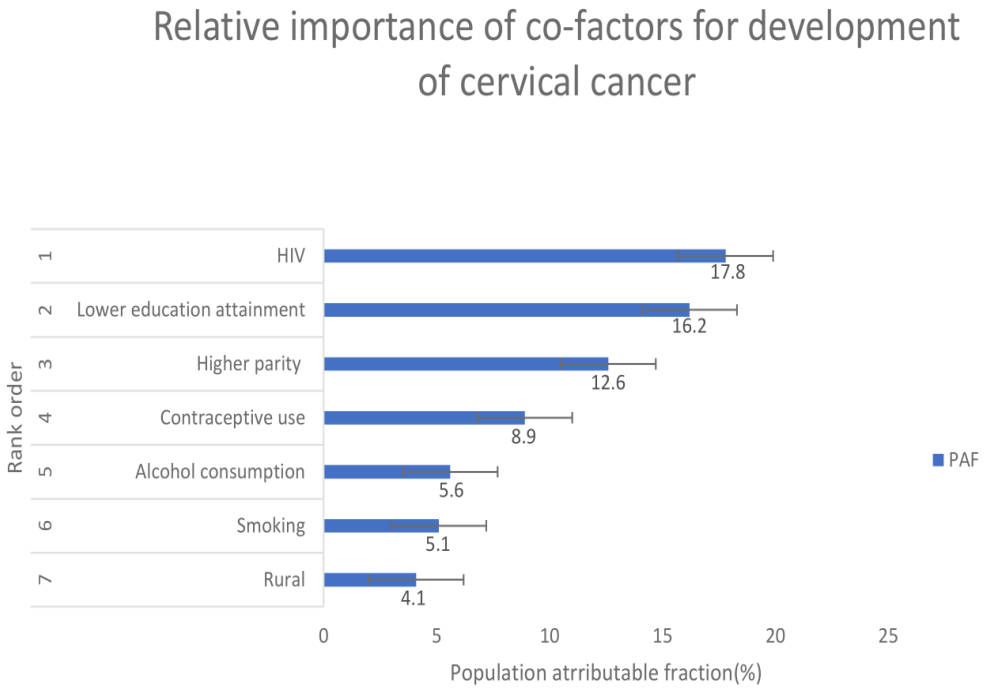


Figure 5-2: Population Attributable Fraction of the Cofactors on Cervical Cancer

We tested the robustness of our control selections on the assumption that the prevalence of exposure for each cancer type chosen in each comparison should be homogeneous. We, therefore, calculated an adjusted (age, number of sexual partners and education level) Mantel-Haenszel Chi-square test of heterogeneity for each type of comparison. We observed no differences ($p > 0.05$) in the prevalence of cofactors across different cancer types in the three control arms except for alcohol Table 5-3.

Table 5-3: Age-adjusted p-value for heterogeneity in the prevalence of cofactors across different cancer types in controls

	Infection unrelated cancer controls			Smoking unrelated cancer controls			Alcohol unrelated cancer controls			Hormonal contraceptives unrelated cancer controls		
Co-factors	*DF	Chi-square	p-value	DF	Chi-square	#p-value	DF	Chi-square	p-value	DF	Chi-square	p-value
Parity	-	-	-	-	-	-	-	-	-	10	9.02	0.417
HIV	9	15.2	0.076	-	-	-	-	-	-	-	-	-
Number of sexual partners	9	11.02	0.274	-	-	-	-	-	-	-	-	-
Smoking	-	-	-	3	5.44	0.142	-	-	-	-	-	-
Alcohol use	-	-	-	-	-	-	8	17.13	0.029	-	-	-
Contraceptive use	-	-	-	-	-	-	-	-	-	15	30.82	0.051

*DF=degrees of freedom, #p-value <0.05

5.4. Discussion

In descending order, cervical cancer was associated with HIV-positive, educational attainment, higher parity, contraceptive use, heavy consumption of alcohol, current smoking and residing in rural areas among Black South African women.

In the current study, cervical cancer risk was significantly associated with HIV infection, ranked as the most important risk factor, with a PAF of 17.6%. The PAF implies that about 18% of cervical cancers would be prevented if individuals were not infected with HIV. This finding is particularly important in South Africa, where Black women are disproportionally affected by HIV compared to other racial groups [166]. Several studies in low support the association between HIV and cervical cancer- and middle-income countries [201,202], showing that during HPV pathogenesis, coinfection with HIV increases HPV viral persistence [202]. Our finding is similar to the previous study on the spectrum of HIV-1-related cancers in South Africa, which used the JCS data. In that study, the risk of cervical cancer was elevated in HIV-1 positive women, OR=1.6 (95% CI: 1.1–2.3) [203]. Similarly, in a Ugandan study, HIV-positive women had an increased risk for cervical cancer (AOR= 2.4, 95% CI: 1.1–4.4) [189]. Efforts to reduce new HIV infections among Black women in South Africa would likely reduce the incidence of cervical cancer in these women in future.

High incidence rates of cervical cancer have been reported among women with low socioeconomic status in high- and low-income settings [1,204]. Socioeconomic status may affect cervical cancer incidence via levels of educational attainment. Education, a measure of socio-economic status, is inversely related to cervical cancer risk [205]. In a study from Spain and Columbia, having no education was associated with cervical cancer (OR=2.5, 95% CI: 1.6 -3.9) [206]. Similarly, our study found that having no education

or completing primary school was associated with an increased risk of cervical cancer. Our results indicate that 16.9% of cervical cancers would have been prevented if women with primary education had secondary and above education. Future investments in education should drive cervical cancer rates downwards.

In our study, higher parity (possibly related to age at first sexual debut and perhaps early exposure to HPV infection) was associated with the risk of developing cervical cancer and a PAF of 12.6%. Our findings are in agreement with Briton et al. [207], Castellsague et al. [187] and Jensen et al. [208], who demonstrated an association between higher parity (2 or more children) and cervical cancer risk. The International Agency for Research on Cancer (IARC) showed a 3.8 (95% CI: 2.7–5.5) times risk for cervical cancer with 7 or more full-term pregnancies [209]. Having 2 or more children was associated with persistent HPV infection, facilitating cervical cancer development [208]. Similar to our study, PAFs of between 24% and 44% were reported for the USA and Italy [210] and 42% in Costa Rica for higher parity [191]. Evidence suggests that the burden of cervical cancer should decrease if average family sizes decrease [209,211].

Prolonged use of hormonal contraceptives has been associated with cervical cancer [212]. A previous study from the JCS demonstrated an association between the duration of hormonal contraceptive use and cervical cancer [193]. Our study did not consider the period of contraceptive use and cervical cancer. Nonetheless, we reported OR=1.48 (95% CI: 1.24-1.77) and a PAF of 8.9%. Our findings, which showed an association between the use of both injectable and oral contraceptives use and cervical cancer, are broadly similar to the JCS findings of Urban et al. [193] (OR=1.38, 95% CI: 1.08-1.77), as well as for injectable only and cervical cancer (OR=1.58, 95% CI:1.16-2.15). Our finding is comparable to that of Appleby et al. [212], which contains earlier JCS data, where combined contraceptive use was associated

with cervical cancer. Similarly, in Latin America, Herrero et al. [213] found an association between injectable contraceptive use and cervical cancer, which reduces significantly after cessation of use. Thus, prolonged use of hormonal contraceptives should be time-limited to avoid excess risks.

The evidence concerning alcohol consumption and the risk for cervical cancer is equivocal. Some studies demonstrated an association with alcohol consumption [190,214,215] but others did not [216,217]. Our study, however, found a significantly elevated risk for cervical cancer in heavy drinkers. Our data on PAF suggest that 5.6% of cervical cancer cases would be avoided if alcohol consumption were reduced. It is possible that women who were heavy drinkers in our study may have also been involved in other high-risk behaviours such as smoking, having multiple sexual partners and other behaviours that promote the acquisition of hrHPV [218]. Notably, the lack of enough data on cervical screening made it difficult to control for it. Thus we cannot preclude the effects of residual confounding.

Current smoking has consistently been associated with cervical cancer pathogenesis. We reported an OR of 1.55 (95% CI: 1.34-1.80) for current smokers and a PAF of 5.1% for smoking. A 2008 JCS study found that current smokers in South Africa had an increased risk of cervical cancers relative to never smokers (OR=1.5, 95% CI: 1.2–1.8) [111]. Similarly, Roula et al. [219], in a European prospective cohort study, showed that current smokers had a Hazard Ratio (HR) of 1.6 (95% CI: 1.4-2.5) for cervical cancer. Besides, a 2011 international study by IARC showed that current smokers compared to never smokers had an OR=1.94 (95% CI: 1.26–2.98) risk of developing cervical cancer [220]. While smoking is a significant risk factor for cervical in other populations, it might not be as important a risk factor in Black South African women, where smoking prevalence is low (4.1%) [221]. Continuing progressive anti-smoking laws and health promotion in South Africa are needed to retain low smoking prevalence among Black females.

We found an association between residing in rural areas and cervical cancer. Our finding is comparable to Yang et al. [222], which showed an association between rural areas and cervical cancer (OR=1.46, 95% CI: 1.03-2.06) and a PAF of 4.1%. This could be due to a lack of cervical cancer screening services, poverty in rural areas, lower education attainment and low uptake in cervical cancer screening. Vhuromu et al. [13] reported low utilization of cervical cancer screening in rural areas. Improved health systems and new methods of cervical cancer screening services in rural areas may increase the uptake of cervical cancer screening. Such efforts would likely reduce cervical cancer cases in rural areas.

Sexual intercourse is the main route for HPV transmission. Having multiple sexual partners increases the risk of HPV infection among women, which may result in cervical cancer. Other studies have demonstrated an association between the number of sexual partners and cervical cancer risk [223,224]. Liu et al. [223] used a meta-analysis of 41 studies. They found that the number of sexual partners was an independent risk factor for cervical cancer even after adjusting for HPV infection. Appleby et al. [113], in an international collaboration of 21 epidemiological studies, demonstrated an association between the number of sexual partners and cervical cancer. Our study did not find an association between the number of sexual partners and the risk of cervical cancer. However, there was a slight increase in risk, which was not significant Table 5-2. The possible explanation for the non-significant finding in our study could be due to the small sample size among women aged 25-34 years, reporting bias (erroneously reporting fewer sexual partners than in reality), confounding by HIV, and no partner data available on male partners.

Our study supports the importance given to the cofactors of cervical cancer and the need for effective interventions of these cofactors. In South Africa, cervical cancer rates could increase or decrease depending on the effectiveness of public health policies. The PAF considers the cofactor's prevalence and adjusted odds ratios, underscoring that an increase in the prevalence of these cofactors could have a notable effect on cervical cancer incidence among Black women in South Africa.

There are some strengths to our study. The larger sample size allowed us to estimate the contribution of key lifestyle cofactors more reliably than before. The selection of appropriate sets of cancer controls unrelated to a specific exposure of interest minimised the bias of the estimates related to the exposure and referral, interviewer and recall biases [196,197]. Both cases and controls were cancer patients and likely to remember their past exposures similarly. Our is the first study to measure the relative importance of a wide range of risk factors for cervical cancer in the same study population by adjusting for a common set of confounders.

This study has several limitations. The effects obtained were not adjusted for the frequency of Pap smear screening since the data available was self-reported, and the question of Pap smear was only added in 2001. We could not adjust for unmeasured confounders despite adjusting for known confounders such as age and HIV status. Since the study only focused on Black South African women, mainly residents in Johannesburg and Soweto, the findings from this study may not be generalizable to the entire population of South Africa. Another limitation of this case-control study is reverse causality, whereby people with long-standing conditions may change their lifestyle. We mitigated this by reclassifying those who reported quitting smoking five years before diagnosis as current smokers in case they altered their habits because of underlying health problems. Although the questions on alcohol consumption were focused on before the participant became ill, we could not perform a similar re-classification as for smoking.

Because different exposures required different control selections, we avoided measuring combined exposures, e.g. smoking and drinking.

Conclusions

In order of importance, HIV positivity, educational attainment, parity, hormonal contraceptive use, alcohol, smoking and residing in rural areas were associated with cervical cancer among Black South African women. These women should be prioritized in opportunistic or planned cervical cancer screening programs. Our findings confirm previously known cofactors of cervical cancer and provide a rank order of risks that could be used locally to target educational messaging and appropriate interventions.

Acknowledgements

We acknowledge the contributions of all authors in the ERICA-SA collaborative for their efforts in providing feedback to improve the final version of the manuscript.

Table S 5-1:List of cancer controls

Cancer type	ICD-O-3-code	Frequency N= 5,709	Infection-unrelated cancer controls	Smoking unrelated cancer controls	Alcohol-unrelated cancer controls	Hormonal contraceptives unrelated cancer controls
Breast	C50	3,843	✓	✓		
Colon	C18-20	340	✓			✓
Oesophagus	C15	314	✓			✓
Ovaries	C56	263	✓		✓	
Endometrium	C54-55	224	✓	✓	✓	
Lung Cancer	C33-34	159	✓			✓
Myeloma	C90	123	✓		✓	✓
Myeloid Leukaemia	ICD-10 C92	105	✓		✓	✓
Pancreas	C25	51	✓			
minor cancers						
Melanoma	C43	39	✓	✓	✓	✓
Larynx	C32	27	✓			✓
Kidney	C64	20	✓			✓
Fallopian tube	C57.0	31	✓	✓	✓	✓
Meninges	C70	1	✓	✓	✓	✓
Endocrine gland	C75	2	✓	✓	✓	✓
Bone	C40-41	33	✓	✓	✓	✓
Brain	C71	14	✓	✓	✓	✓
Central nervous system (CNS)	C72	1	✓	✓	✓	✓

Cancer type	ICD-O-3-code	Frequency N= 5,709	Infection-unrelated cancer controls	Smoking unrelated cancer controls	Alcohol-unrelated cancer controls	Hormonal contraceptives unrelated cancer controls
Peritoneum and retroperitoneum	C48	9	✓	✓	✓	✓
Placenta	C58.9	33	✓	✓	✓	✓
Small Intestine	C17	9	✓	✓	✓	✓
Soft Tissue Sarcoma	C49	26	✓	✓	✓	✓
Thymus	C37	5	✓	✓	✓	✓
Thyroid	C73	37	✓	✓		

PART 3

GENERAL DISCUSSION

and

CONCLUSIONS

6.0. Chapter Six: General Discussion

6.1. Introduction

The chapter starts by presenting the key findings of the PhD thesis in Table 6-1. The chapter then reflects on research themes that emerged from these studies. It clarifies how these can inform the 90-70-90 global cervical cancer elimination strategy. The chapter also focuses on the role of HPV antibody testing as a screening tool or point-of-care testing. It further provides additional comments on the ranking strategy of cervical cancer risk factors to improve cervical cancer education strategies. The chapter reflects on the conceptual framework's utility and methodological considerations (limitations and strengths). This chapter ends by providing some recommendations for future research.

6.2. Summary of key findings of the PhD thesis

Table 6-1: Summary of key findings

Chapter	Objective	Key findings
3	To investigate the utility of antibodies to HPV-16 and -18 (E6 and E7) in cervical cancer screening.	There is limited literature on E6/E7 as a biomarker for ICC and CIN2/3. HPV-16 and -18 E6 and E7 antibodies had high specificity but low sensitivity to detect cases with cervical cancer or CIN2/3 precancerous lesions.
4	To calculate the seroprevalence of antibodies to HPV-16 and -18 (L1 and E6 and E7) proteins among infection-unrelated cancer controls by socio-demographics and sexual history and assess their association with HIV.	In HIV-negative controls, HPV-16 E7 and HPV-18 E7 seroprevalence differed by place of residence.

Chapter	Objective	Key findings
		Among HIV-positive women, HPV-16 E6 & E7 seroprevalence increased with the number of births.
4	To assess the association between HPV-16 and -18 (E6 and E7 and L1) and cervical cancer in HIV-positive and HIV-negative patients.	HPV-16 and -18 (E6 and E7) oncoproteins were associated with cervical cancer risk in HIV-positive and HIV-negative patients. HIV-positive patients, compared to HIV-negative patients, had lower sensitivity but high specificity for HPV-16 and HPV-18 (E6 and E7)
5	To evaluate the relative contribution of several known lifestyle co-risk factors for cervical cancer among urban Black South African women.	Cervical cancer, in decreasing order, was associated with being HIV-positive, having low educational attainment, higher parity, contraceptive use, heavy consumption of alcohol, current smoking and residing in rural areas.

6.3. Research themes that emerged from the findings.

The following are the emerging themes based on the consolidated findings of the research: (1) HPV-16 and -18 E6 and E7 antibodies as a screening test, (2) current evidence on E6/E7 antibodies and ICC and CIN2/3 detection, (3) HPV-16/18 E6 and E7 seropositivity and place of residence, (4) HPV-16/18 E6 and E7 antibodies and Cervical Cancer by HIV status and (5) a ranking strategy of cervical cancer and educational messaging/ appropriate interventions.

6.3.1. HPV-16/18 E6 and E7 antibodies as a screening test

Specificity plays an important role in screening assays as it reduces the errors that might occur due to the false-positive- values that could lead to overtreatment [225]. The systematic review and meta-analysis in Chapter 3 highlighted the utility of HPV-16 and -18 E6 and E7 antibodies in cases with cervical cancer or CIN2/3 precancerous lesions. Thus, sensitivity values are likely too low to translate these biomarkers into an effective screening tool. Nonetheless, in Chapter four, this study showed that the current sensitivity and specificity values among HIV-negative women were similar to those observed with Pap smears (with strong caveats). Therefore, if these values improve, there may be some potential for using some similar approach for screening or earlier detection in places w no screening. As the JCS is essentially cross-sectional (antibodies measured at the time of cervical cancer diagnosis), ideally, data from a cohort study are needed to measure multiple hrHPV antibody levels before the diagnosis of CIN2/3 or worse.

6.3.2. Current evidence on E6/E7 antibodies and ICC and CIN2/3 detection

While HPV-16 and -18 E6 and E7 antibodies may be promising biomarkers for the detection of cervical cancer cases, studies are still very few studies on the relationship between HPV E6 and E7 antibodies and cervical cancer [59,136,149]. In addition, the evidence synthesis yielded too few papers to evaluate antibodies as a screening or point-of-care test. Despite limited literature on the subject matter, in Chapter three, the available evidence about the utility of HPV E6/E7 antibodies in detecting cases of cervical cancer was synthesised.

6.3.3. HPV-16/18 E6 and E7 seropositivity and place of residence

The prevalence of HPV in women varies by geographical region and country [226]. In South Africa, rural-urban differences in access to healthcare remain a challenge across provinces [227]. In rural South Africa, cervical cancer screening remains challenging due to the lack of healthcare services, mainly found in urban areas [172]. In our study, we found that among HIV-negative controls, women who resided in rural areas had higher HPV-16 E7 and HPV-18 E7 antibodies seroprevalence than women who lived in urban areas (Chapter four). Our finding confirms the vital role of place of residence as a risk factor for HPV prevalence.

6.3.4. HPV-16/18 E6 and E7 antibodies and Cervical Cancer by HIV status

Previous studies on HPV antibodies and HIV have mainly focused on past and current HPV exposure [81]. To successfully translate HPV biomarkers into a point-of-care testing tool, they must be extensively validated, including in populations with a high prevalence of HIV in LMICs and especially in Africa [228]. However, most studies that investigated the association between HPV E6/E7 antibodies and cervical cancer were in settings with low-HIV prevalence [59,91]. Two case-control studies of women from Columbia and Spain that used ELISA assay and RIPA showed that HPV-16 E6 and E7 biomarkers were associated with invasive cervical cancer [229,230]. We found that HPV-16 and -18 E6 and E7 antibodies were associated with cervical cancer in HIV-positive and HIV-negative patients. Peculiarly, the association between HPV markers and cervical cancer in HIV-positive women was weaker, so it could be hypothesized e that other HPV types may be responsible for this group of women. If this is correct, HIV-positive women may benefit more if screening tested for a broader range of HPV types and if they had access to a multivalent hr HPV

vaccine. Nonetheless, this study has provided new evidence on the role of HPV E6/E7 antibodies in discriminating cervical cancer cases from controls in a Black South African population. Future serological studies encompassing the other main circulating hrHPV types would be informative.

6.3.5. A ranking strategy for cervical cancer and educational messaging/ appropriate interventions

The importance of cervical cancer lifestyle cofactors varies in prevalence (and possibly the risk) in different settings (Chapter five). In settings where HIV- prevalence is high, a high burden of cervical cancer has been reported [231]. In South Africa, besides the double burden of HIV and cervical cancer, coverage for cervical cancer screening is low [29]. In people living with HIV, the risk of HPV infection and persistence increases at least 2 fold [177]. In our study, among Black South African women, HIV was ranked first as an attributable factor to a high burden of cervical cancer (Chapter five). Thus, prioritisation of prevention strategies to reduce cervical cancer should be among women living with HIV. In addition, reducing new HIV infections would help reduce the incidence of cervical cancer.

In South Africa, there have been historical disparities in levels of educational attainment among the Black women population [232] (Chapter five). Illiteracy and lower levels of education are indicators of poverty [233]. We found that lower educational attainment was the second contributing factor to the higher burden of cervical cancer among Black women (Chapter five), as seen in other studies, e.g. Mabotja [233]. Furthermore, paper three (Chapter five) provides a detailed ranked order of several major lifestyle risk factors. In summary, this study offered novel ways of cancer epidemiology in an urban South African setting by systematically selecting controls and ranking

the cofactors of cervical cancer in their order of importance to inform primary and secondary prevention strategies.

6.4. Reflection on the WHO-global strategy for cervical cancer elimination - the 90-70-90 targets

6.4.1. HPV Vaccination

The WHO global cervical cancer elimination strategy states that 90% of girls should be fully vaccinated with the HPV vaccine by age 15. The ultimate goal is to eliminate cervical cancer, mostly in low and middle-income countries, by 2030 [132]. The long-term benefits of HPV vaccination show up in HICs, with many women vaccinating against HPV. For example, in Australia, significant progress is expected toward reaching the elimination target of fewer than 4 cases per 100,000 women by 2035 due to the high coverage of national screening and vaccination programs [234]. In contrast, very few women (1%) had received HPV vaccination from LMICs by 2014 [235], which makes a majority of women from LMICs remain at risk of cervical cancer.

6.4.2. Screening

The second ambitious global target is that 70% of women should be screened with high-throughput tests by the age of 35 years and again by the age of 45 years. The key to achieving these ambitious targets is population-based cervical cancer screening programmes. In 2019, according to a survey by WHO, findings showed that of the 194 countries, only 65% had country screening programs,

62% reported organised population-based screening programs and were from HICs [236]. However, screening coverage in the African region ranged between 10 to 50% [236]. WHO has highlighted research on less invasive (serological tests), and with advances in the development of rapid antibody tests, such tests could help to scale up screening rates by 2030. For example, in LMICs, using an HPV serology screening test or a point-of-care test could potentially assist in detecting cervical cancer in places where screening is unavailable [132].

Precancerous lesions of the cervix are easier to treat, with a cure rate of at least 90% compared to invasive cancer of the cervix. Even within invasive cancer, survival is much higher in early-stage cervical cancer (i.e. Stages 1 and 2a) when compared to stage 2b or worse [237]. So serological tests' role in detecting earlier invasive cervical cancer lesions needs further evaluation [238]. In addition, screening cannot occur without the infrastructure to treat women with abnormal results adequately. A 2019 survey by WHO [236] found that 30% of LMICs, compared to 90% of HICs, had recommended diagnostic and treatment infrastructures in public health hospitals. However, in LMICs, where adequate treatment facilities are available [233], a case can be made for an accurate finger-prick-based serological screening test constructed to work at room temperature and withstand temperature variations. Infrastructure set-up, purchase and training costs for such a test would be low. By comparison, other screening methods (visual inspection of the cervix using acetic acid, Pap or HPV-based screening tests) require significant resources to develop such laboratory and referral infrastructure, hire specialist staff and retain high-quality assurance.

6.5. The role of HPV serology test in the cervical cancer global elimination strategy

Since South Africa has committed to the 90-70-90 cervical cancer global elimination strategy, achieving these ambitious targets stems from many factors, including new screening tests. Suppose the sensitivity of HPV serology markers improves. In that case, such HPV-serology tests could reach women in a manner that conventional cervical screening methods cannot do. Hence, the HPV-serology test could potentially contribute to the second strategy (70%) of the global targets for cervical cancer elimination by providing a less invasive option for screening women unwilling or unable to go to cervical cancer screening facilities. In addition, the HPV antibody test could be a screening tool in settings with limited infrastructure and gynaecologists to screen women for cervical cancer.

Critically, before its rollout, there shall be a need for feasibility studies, i.e. mathematical modelling, to evaluate the impact of the HPV serology test in scaling uptake. Also, health economic modelling to assess the cost-benefit analysis of such an HPV-serology test in a high-risk population to improve the scale-up of the HPV serology test. Wilson and Jungner [69], in the principles and guidelines of disease screening, also proposed a cost-benefit analysis before implementing a screening program. In addition, it is crucial to consider the test's positive predictive value. Table 6-2 illustrates a worked example using hypothetical data of what a predictive value of a positive test may look like in a high prevalence setting. For instance, in an environment of cervical cancer incidence of 500/1,000,000 women, an HPV serology screen test with a sensitivity of 43% and specificity of 91% would yield a positive predictive value (PPV) of 0.24%. The PPV translates to approximately 400 cases missed for every case detected. Therefore, a need for additional (triage) tests

with higher PPV; if not, then adjusting the cut-off values of the HPV antibody test to increase its sensitivity. Thus a realistic approach to cervical cancer elimination would be investing in resources such as strengthening healthcare systems, funding for cervical cancer research, training the needed workforce, and a well-described surveillance mechanism for screening and infrastructures in the limited resource settings. Furthermore, the achievement of these goals are country specific; for example, different countries within the same region have different guidelines on cervical cancer screening.

Table 6-2: Estimated Positive Predictive values in a setting with higher incidence rates of cervical cancer

	Disease Status			
Serology test	True+	True-	**Total**	**Predictive values (PV)**
Test+	215	89,955	90,170	PV+=0.24%
Test-	285	909,545	909,830	PV-=99.96%
Total	500	999500	1,000,000	

6.6. Way forward to improve cervical cancer education strategy

Health education is critical for raising awareness and motivating women to attend cervical cancer screening programmes [239,240]. With the proper knowledge of cervical cancer risks, women are more likely to make informed decisions to participate in cervical cancer screening [241]. The health belief model has been used in different settings to encourage women to attend cervical cancer screening [240,241]. The health belief model states that women's quick decision-making to seek health care results from their perceived risk of the disease, perceived seriousness of the condition,

perceived benefits, and outcomes [242]. Figures 6-1 illustrate how the health belief model can be used to promote health education among women that could lead women to make decisions to attend cervical cancer screening. However, other issues like accessibility of screening services, their price and associated costs and staff attitudes can still discourage women from screening for cervical cancer.

Through health promotion efforts, women are more likely to understand the perceived seriousness of cervical cancer and its consequences and participate in cervical cancer screening. In South Africa, Mabotja et al. [243] used the health belief model amongst women attending primary care in Johannesburg. They found that women who perceived cervical cancer as a severe condition were more likely to participate in Pap smear screening. In Lagos, Nigeria, Wright et al. [244] found that community-based campaigns and customarily sensitive information were better education interventions for reaching out to the high-risk population. They also increased knowledge about cervical cancer screening. In their systematic review, Naz et al. [240] reported that education methods such as radio broadcasting, small group discussion, and consultation were some effective interventions in changing women's behaviour to participate in cervical cancer screening. In rural Nigeria, Abiodun et al. [245] found that educational movies improved rural women's knowledge about cervical cancer screening.

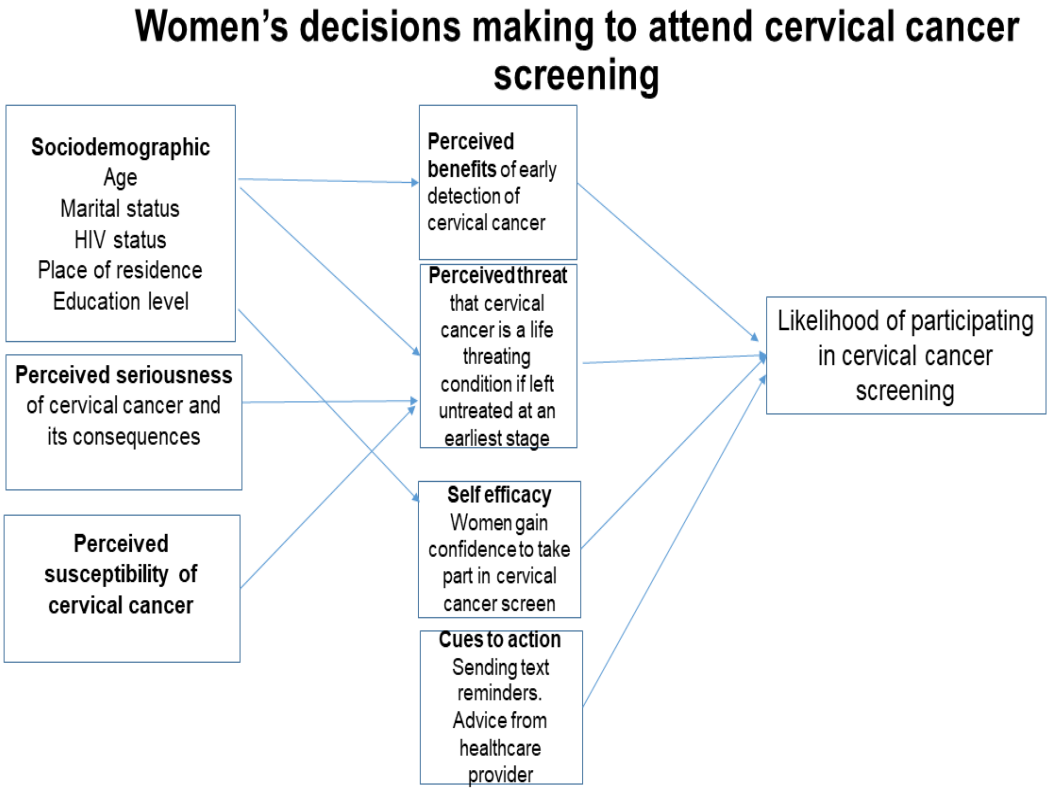


Figure 6-1: Using the Health Belief Model for cervical cancer education strategy adapted from Becker [242].

6.7. The utility of the conceptual framework

The conceptual framework used in this PhD thesis was chosen based on the aetiology of cervical cancer and the interplay of the cofactors in the pathogenesis of cervical cancer. It was mainly driven by the critical literature and the overall aim of the study. It assisted in the identification of critical determinants of the research and their relationship to cervical cancer.

6.8. Methodological considerations

This part discusses some methodological considerations regarding this study. It mainly discusses the strengths and limitations of the study and highlights some potential biases.

6.8.1. Strengths

The main strength of the PhD is the blending of a systematic review of serological and lifestyle data to answer an important public health question. The other strength is the JCS, with at least 26,000 Black South African patients recruited with newly diagnosed cancers. It's the largest study in Africa to assess the role of key lifestyle cofactors, including HIV, contraceptive use, smoking, alcohol use and parity, more reliably. The use of a multiplex immunofluorescent HPV serology assay conducted by a laboratory without prior knowledge of the case-control status of the specimens is another necessary strength of this study. A further advantage of a multiplex serological assay is that it can analyse different antigens in a single experimental run. Most studies on HPV seroepidemiology have used an ELISA, which analyses a single antigen at a time.

6.8.2. Limitations

The main potential weaknesses of case-control studies relate to estimates of past exposure (recall biases), interviewer bias, referral biases, and reverse causation. This section discusses ways the JCS and the current analyses attempted to minimise these biases are discussed: Regarding recall bias, the JCS focused on questions about lifestyle and exposures where recall bias is likely to be minimal. The JCS focused on questions where previous case-control studies produced reliable answers (in this case, smoking, alcohol contraception, sex partners, place of residence, and parity).

Further, both cases and controls were patients with cancers (of similar disease severity), so they are likely to remember their past exposures similarly. Likewise, interviewers were not consciously aware of the different hypotheses at play at any point in time, so any interviewer biases are likely to be non-differential.

With both cases and potential controls being people with cancer, we minimised referral biases because all people with cancer travel through a similar referral pathway. We routinely control for urban/rural status to minimise referral biases as much as possible. Further, over 80% of eligible patients participated in the study; most non-responders were too ill to be interviewed [194], so the chances of non-response bias re again minimised. Another bias in retrospective case-control studies is reverse causality, whereby people with long-standing conditions may change their lifestyle. In our research, we mitigated this potential bias by reclassifying those who reported quitting smoking five years before diagnosis as current smokers in case they altered their habits because of underlying health problems. The questions on alcohol consumption were focused on before the participant became ill. However, we could not perform a similar re-classification for smoking. Our study results are based on Black women aged 25-64 years recruited from the catchment area of the main tertiary hospital in Johannesburg. Therefore, our findings might not be generalizable to other South African regions and populations. The few studies included in our meta-analysis in Chapter three compromised precision. Due to the different assays, we could not pool all the studies together.

The HPV-16 and -18 proteins serology study described in Chapter four lacked HPV DNA cervical samples to assess the effect of the other hrHPV types. A large proportion of the older women did not have serology samples available. Therefore, our findings of the serology study could not be

generalised to women aged 55 years and above. The JCS did not collect the stage of cervical cancer at diagnosis- – if E6/E7 expression is related to cervical cancer severity, the sensitivity of our results may be overestimated. Furthermore, studies need to measure the utility of these HPV markers in detecting early-stage cervical cancer. In addition, sensitivity for the biomarkers was even lower in HIV-positive patients. Future research options to improve the sensitivity include incorporating additional biomarkers [246], including other HPV serotypes [10] or re-examining current cut-off values for positivity.

Our study assessed only two hrHPV types for HPV L1 E6 and E7 antibodies. Other common hrHPV types in South Africa are HPV31, HPV33, HPV35, HPV52 and HPV58 [95]. However, our study did not assess the seropositivity of such types due to the unavailability of serology data. Nonetheless, Combes et al. [91] reported that E6 and E7 antibodies seroprevalences of 66.1% for E6 and E7 antibodies in cervical cancer cases (from India and Algeria) for combined HPV types 16,18,31,33,35,45,52 and 58. In addition, in Korea, HPV antibody seropositivity in cervical cancer was 10.7% for L1, E6 (14.3%) and E7 (26.8%) for HPV58 [93]. However, in different settings, low seroprevalence ranging from 0.5% to 10% of anti- HPV- 16 and -18 E6 and E7 antibodies have been detected among HPV cervical cancer controls [83,91,93,165]. Similarly, we observed the same range of seroprevalence for HPV- 16 and -18 (E6 and E7) antibodies among controls.

In the lifestyle risk factors for cervical cancer study in Chapter five, it would have been helpful to obtain effects adjusted for the frequency of Pap smear screening. However, the available data was self-reported. In addition, the JCS only added the question of Pap smear in 2001 (screening coverage was very low, and some participants may have misinterpreted diagnostic workup tests as a 'Pap

test'). We could not control unmeasured confounders despite adjusting for known confounders such as age and HIV status. Most of the study population was from an urban area. Therefore, the rank order of risks (e.g., living in a rural area) will likely change if the study setting changes to a rural area. We did not find a statistically significant relationship between the number of sexual partners and cervical cancer risk. The possible explanation for the non-significant finding in our study could be due to the small sample size among younger women aged 25-34 years, reporting bias (erroneously reporting fewer sexual partners than in reality), confounding by HIV, and no partner data available on male partners.

6.9. Future Research

Based on the findings of this study, We identified the following research gaps.

a. Confirm the role of antibodies to other hrHPV types in detecting cervical cancer, especially in HIV-positive and negative individuals.
b. Measure the utility of antibodies to hrHPV markers in detecting earlier-stage cervical cancer.
c. Evaluate and validate the seroprevalence of HPV protein antibodies in the high-risk population.
d. Investigate the role of HPV antibodies in other anogenital and pharyngeal cancers.
e. Identify markers in addition to HPV that could improve the detection of pre-cancerous lesions by including, for example, Genome-Wide Association data to develop a polygenic risk score for cervical cancer (however, the current costs are prohibitive).

f. Identify actionable predictive value cut points and perform whole-of-program cost-benefit analyses of novel point-of-care or cervical screening tests.

6.10. Policy Implications and Recommendations

Early detection of cervical cancer is important for policy, public health practice and advancement in research for numerous reasons. Early detection of cervical cancer helps detect premalignant lesions at an earlier stage. It prevents deaths due to invasive cervical cancer. Increasing uptake for cervical cancer screening should target the use of screening tools that are least invasive and culturally acceptable among women in low-and middle-income countries. This current study has highlighted the importance of HPV antibodies as a screening tool for women in low-and middle-income countries. Since less than one per cent of women with persistent HPV infection develop cervical cancer, interventions should focus on addressing preventive strategies for other risk factors to increase women's awareness.

Our findings have implications regarding research, policy and public health practice. In terms of research, there is a need for more studies, especially population-based cohort studies, to investigate the feasibility and cost-effectiveness of these biomarkers as a point-of-care or screening tool. More studies are needed in high-risk HIV populations to evaluate the sensitivity and specificity of these biomarkers.

In terms of policy, this PhD provides further evidence that assessing HPV E6 and E7 antibodies alone is insufficient. Still, with improvements, HPV E6 and E7 could become potential serology

markers for screening cervical cancer that would help reduce cervical cancer incidence. As mentioned in Chapter three, incorporating such a test into population screening using point-of-care testing of appropriately aged non-vaccinated women could improve screening rates and reduce losses to follow-up. In addition, the test could ultimately lead to earlier detection of the disease, downstaging clinical presentations and reduced cervical cancer incidence rates in limited-resource settings. Critically, with the available evidence use of serology biomarkers as a screening tool in a high-risk population would return a large proportion of false negatives. Based on the findings of this PhD, the question still stands; will the use of HPV antibodies as a screening tool for cervical cancer be achieved in the near future?

In addition, the ranking strategy of cofactors from this PhD would help policymakers and practitioners develop local practical interventions. Such efforts would help to improve cervical cancer education literacy among women at risk to prevent cervical cancer.

7.0. Chapter Seven: Conclusions

The systematic review and meta-analysis for the thesis found a minimal number of studies that assessed the role of HPV-16 and -18 oncoprotein antibodies in the detection of cervical cancer. In a high HIV prevalence setting, the JCS study provided a unique opportunity to add to knowledge on HPV-16 and -18 protein antibodies and their relation to cervical cancer in HIV-positive and negative participants. The study also showed that antibodies to HPV-16 and -18 E6 and E6 proteins have low sensitivity but high specificity in detecting cervical cancer. Therefore, not yet a suitable solution for rapid point-of-care or population screening. The important contribution of this PhD includes identifying a screening tool for use in a place where there is no infrastructure and a gynaecologist to screen women for cervical cancer.

Furthermore, this thesis used a case-control approach using data from a large cancer study in Johannesburg. It demonstrated that with a careful selection of controls and appropriate case-control comparisons. In addition, this PhD showed a rank order of lifestyle risk factors for cervical cancer. The health facilities could use it to target education and appropriate messaging of higher-risk groups.

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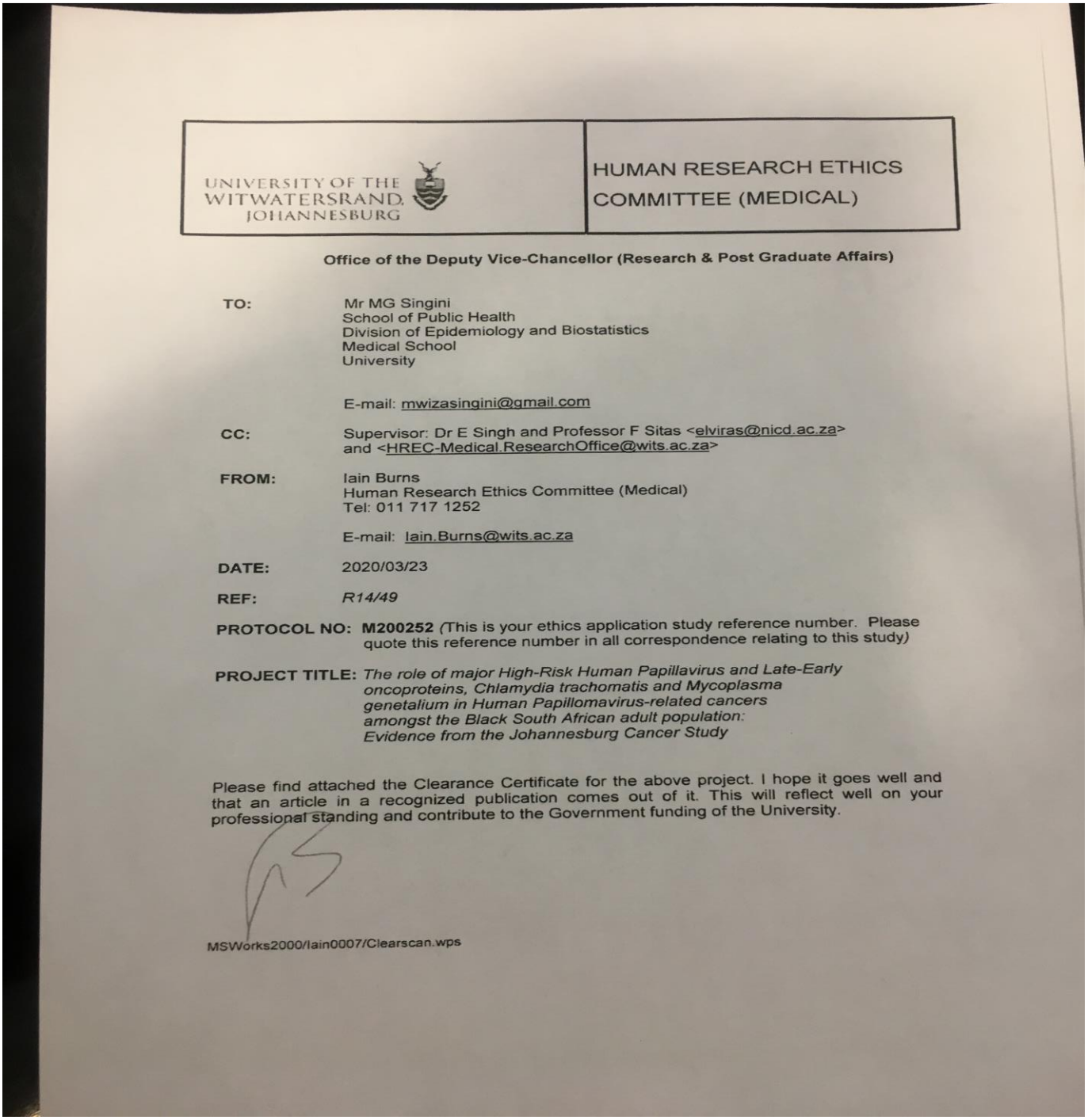
8.0 Appendices

a. Appendix 1: Ethics Certificate



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Mr MG Singini
School of Public Health
Division of Epidemiology and Biostatistics
Medical School
University

E-mail: mwizasingini@gmail.com

CC: Supervisor: Dr E Singh and Professor F Sitas <elviras@nicd.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2020/03/23

REF: *R14/49*

PROTOCOL NO: **M200252** *(*This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study*)*

PROJECT TITLE: *The role of major High-Risk Human Papillavirus and Late-Early oncoproteins, Chlamydia trachomatis and Mycoplasma genetalium in Human Papillomavirus-related cancers amongst the Black South African adult population: Evidence from the Johannesburg Cancer Study*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

R14/49 Mr MG Singini

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200252

NAME: Mr MG Singini
(Principal Investigator)
DEPARTMENT: School of Public Health
Division of Epidemiology and Biostatistics
Medical School
University

PROJECT TITLE: The role of major High-Risk Human Papillavirus and Late-Early oncoproteins, Chlamydia trachomatis and Mycoplasma genetalium in Human Papillomavirus-related cancers amongst the Black South African adult population: Evidence from the Johannesburg Cancer Study

DATE CONSIDERED: 28/02/2020

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr E Singh and Professor F Sitas

APPROVED BY: CB Penny

Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2020/03/23

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and will therefore reports and re-certification will be due early in the month of **February** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

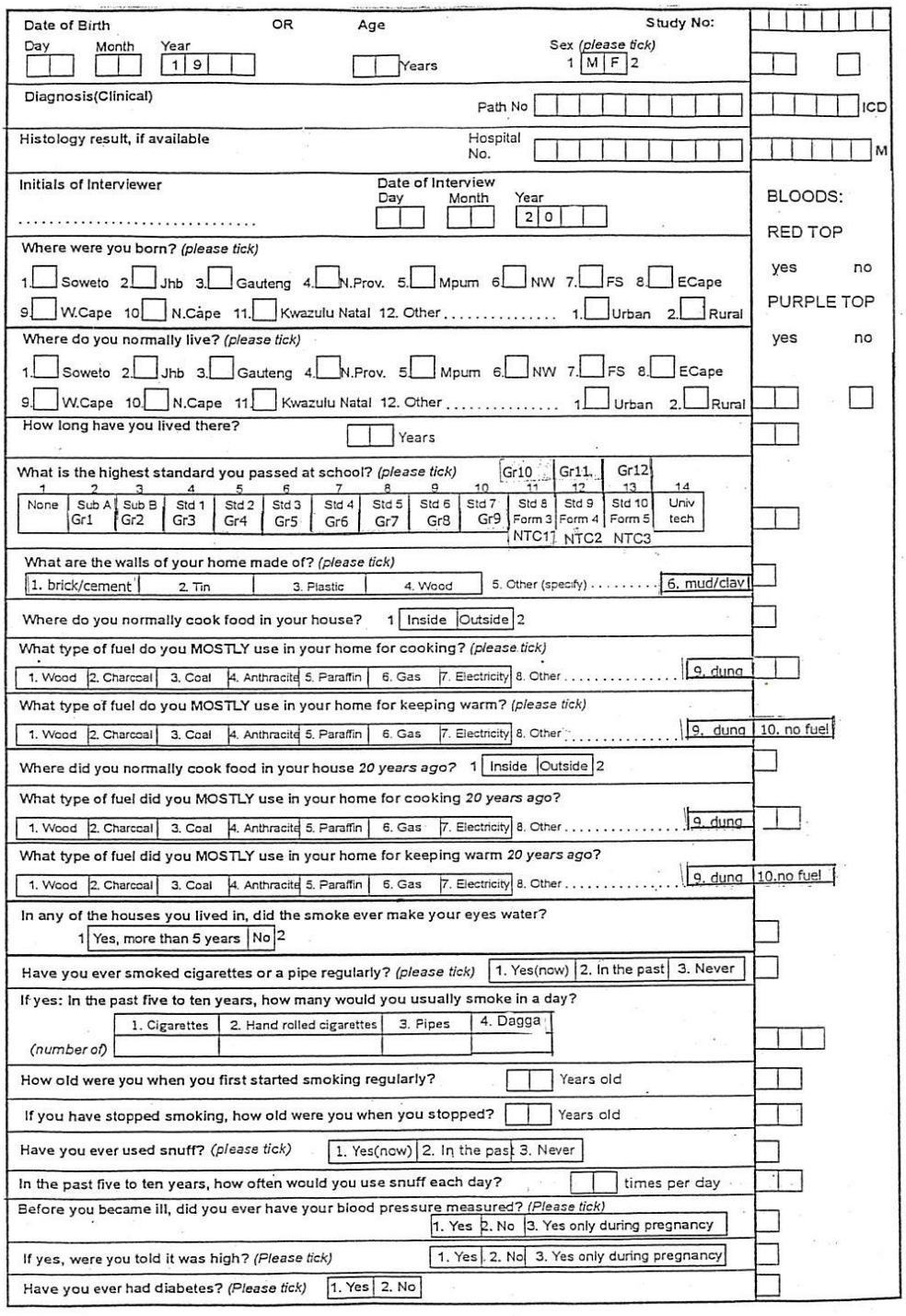
Principal Investigator Signature

27/03/2020
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

b. Appendix 2. Questionnaire for the JCS study

MRC/NHLS/Wits CANCER EPIDEMIOLOGY RESEARCH GROUP (CERG)
JOHANNESBURG STUDY QUESTIONNAIRE FOR PATIENTS

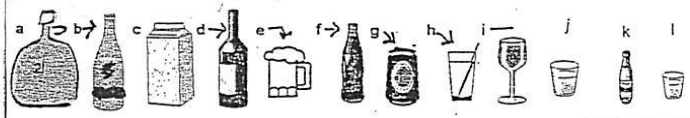


22 January 2010

Before you became ill, about how much wine, beer or spirits did you drink on average each week?
(Please indicate glass/bottle size when you select block for each type of drink - eg. 3 large glasses of maize beer per week would be 3 e if none then 0

BEER			SPIRITS		WINE	CIDER; other	OTHER
sorghum (cartoon)	homemade (specify type)	commercial	Homemade	commercial		commerical alcoholic fruit drinks	SPECIFY

a: 2l (wine bottle)
b: bottle (1 litre)
c: 1l carton
d: 750ml (wine bottle)
e: 500ml (beer glass)
f: 375ml (half jack/pint)
g: 340ml (beer can/dumpy)
h: 250ml glass
i 200ml (wine) glass/nip
j : 100ml (small glass)
k 50ml (miniature airplane bottle)
l 25ml (tot)
mother (please specify)



Are you? *(please tick)*
1. ☐ Single (never been married) 2. ☐ Married(living as married) 3. ☐ Widowed 4. ☐ Separated

How many husbands/wives have you had?

FOR WOMEN ONLY: Have you ever been pregnant? *(please tick)* 1 Yes No 2

If yes, how many times have you been pregnant? ☐☐

If yes, how many times have you had a miscarriage? ☐☐

If yes, how many of your children were born alive? ☐☐

FOR MEN ONLY: How many children *(dead or alive)* do you have?

ALL: How many mothers / fathers do the children have?

ALL: How old is your oldest child now? *(include dead & alive)* ☐☐ Years old

ALL: How old is your youngest child now? *(include dead & alive)* ☐☐ Years old

ALL: How many boyfriends/girlfriends have you had?

FOR WOMEN ONLY
How old were you when your periods began? ☐☐ Years old

Age now (from previous page) _____

Age at first birth _____

Age at last birth _____

Have your periods ended? Yes ☐1 No ☐2 Not sure ☐3 If yes, how old were you when they ended? ☐☐ years old

If Yes, did your periods stop: ☑

1. naturally	2. due to surgery	3. due to medical treatment	4. due to IC use	5. other	6. unknown

	Oral	Injectable
Have you ever taken contraceptives?	1 Yes No 2	1 Yes No 2
If yes, how old were you when you started taking them?	☐☐	☐☐ Years old
How old were you when you stopped taking them?	☐☐	☐☐ Years old
If yes, for how long in total did you take them?	☐☐	☐☐ Years
Interruptions? ☑	1 Yes No 2	1 Yes No 2

Main reason for stopping, the last time you stopped using the method: ☐☐ ☐☐

1 Nausea 2 high BP 3 headaches 4 wt.Gain 5.changed menstrual pattern 6. menopause 7.sterilization 8.hysterectomy 9. no partner 10. to become pregnant 11 other 0.no specific reason

Have you ever had a 'pap' smear?☑

0. Never	1. Yes	2. current illness only	9. Can't remember		If yes, how many?	Age at first pap(yrs)	Age at last pap (yrs)

FOR ALL: Describe your usual or past **occupation** (eg. Driver, machine operator).....

Describe what your usual **workplace** does or did (eg. A bank, a chemical factory, a gold mine).....

What language do you speak most at home? ☐

What language does/did your father speak? ☐

What language does/did your mother speak? ☐

1. Zulu 2. Xhosa 3. Sotho 4.Pedi 5. Tswana 6. Venda 7. Swazi 8. Shangaan 9. Ndebele
10 English 11. Afrikaans 12. Other

22 January 2010

c. Questionnaire for HIV status and ARV use

Study number: _____

Cancer Epidemiology Research Group **Case-Control Study**

Antiretroviral Drug Usage

*Please ask **ALL PATIENTS** the following questions AFTER you have completed the standard questionnaire. Make sure that you emphasize that the information is voluntary and confidential.*

Have you ever been tested for HIV (before current test)? **Yes** $_1$ **No**$_0$ (circle one)

If YES, proceed: Were you tested**: before this illness**$_1$ / **at time of current illness only**$_2$ (circle one)

Were you given the test results? **Yes**$_1$ **No**$_0$ (circle one)

If YES, proceed: Are you willing to disclose your status? **Yes**$_1$ **No**$_0$ (circle one)

If YES, proceed: Are you HIV: **positive**$_1$ **negative**$_0$ (circle one)

Date of last/current test (mm/yyyy): __________

If POSITIVE, proceed:
Have you ever taken anti-Retroviral medicines for your HIV infection? **Yes**$_1$ **No**$_0$ (circle one)

(Be careful to explain to the patient that we mean "Western" medicine/pills/capsules specifically for the HIV itself, NOT for opportunistic infections.)

If YES, Proceed:

From: _____ (start date) To: _____ (end date)

From: _____ (start date) To: _____ (end date)

From: _____ (start date) To: _____ (end date)

The ARV drugs were provided by (tick all that apply):

☐ Private practitioner$_1$ ☐ Public hospital/clinic$_2$

☐ NGO$_3$ ☐ Study/ clinical trial$_4$

d. Appendix 3: Protocol for Systematic Review

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

The usefulness of high-risk HPV early oncoprotein serological markers in the detection of cervical cancer: A systematic review and meta-analysis

Mwiza Gideon singini, Thendo Ramaliba, Melitah Motlhale

Citation

Mwiza Gideon singini, Thendo Ramaliba, Melitah Motlhale. The usefulness of high-risk HPV early oncoprotein serological markers in the detection of cervical cancer: A systematic review and meta-analysis. PROSPERO 2020 CRD42020206036 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020206036

Review question

Are anti-HPV-antibodies especially of HR-HPV16/18 E6 and E7 serological markers useful in the detection of cervical cancer in high and low cervical incidence settings?

Searches

MEDLINE/ PubMed and EMBASE (OVID).

Types of study to be included

Cross-sectional, retrospective case-control or prospective cohort (or nested case-control) and diagnostic accuracy-test studies in which the HPV 16/18 E6 and E7 serological markers were compared to a cytopathology reference standard.

Condition or domain being studied

Cervical cancer constitutes a public health problem globally. In 2018, approximately 590, 000 incident cases (1) and 311. 365 deaths of cervical cancer occurred worldwide. The majority of cervical cancer incident

e. Original Papers:

Received: 21 April 2022 | Accepted: 28 May 2022
DOI: 10.1002/jmv.27900

REVIEW

JOURNAL OF MEDICAL VIROLOGY WILEY

Usefulness of high-risk HPV early oncoprotein (E6 and E7) serological markers in the detection of cervical cancer: A systematic review and meta-analysis

Mwiza Gideon Singini[1,2] | Elvira Singh[1,2] | Debbie Bradshaw[3] | Thendo Ramaliba[3] | Wenlong Carl Chen[1,4] | Melitah Motlhale[1,2] | Abram Bunya Kamiza[4] | Chantal Babb de Villiers[5] | Mazvita Muchengeti[1,2,6] | Christopher G. Mathew[4,7] | Robert Newton[8,9] | Noemi Bender[10] | Tim Waterboer[10] | Freddy Sitas[3,11,12]

[1]National Cancer Registry, National Health Laboratory Service, Johannesburg, South Africa
[2]Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[3]Burden of Disease Research Unit, South African Medical Research Council, Cape Town, South Africa
[4]Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[5]Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[6]South African DSI-NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), Stellenbosch University, Stellenbosch, South Africa
[7]Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College London, London, UK
[8]MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda
[9]University of York, York, UK
[10]Division of Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[11]Centre for Primary Health Care and Equity, School of Population Health, University of New South Wales Sydney, New South Wales, Sydney, Australia
[12]Menzies Centre of Health Policy, School of Public Health, University of Sydney, Camperdown, New South Wales, Australia

Correspondence
Mazvita Muchengeti, National Cancer Registry, National Health Laboratory Service, Sandringham, Johannesburg, South Africa.
Email: MazvitaM@nicd.ac.za

Funding information
UK Government's Newton Fund; South African Medical Research Council

Abstract

We reviewed the literature on the importance of selected anti-high-risk human papillomavirus (HR-HPV) antibodies (namely, 16/18 and early oncoproteins E6 and E7) as potential serological markers for early detection of individuals at high risk of cervical cancer. We searched for studies in PubMed and Embase databases published from 2010 to 2020 on antibodies against HR-HPV E6 and E7 early proteins and cervical cancer. Pooled sensitivity and specificity for HPV16 and HPV18 antibodies were calculated using a bivariate hierarchical random-effects model. A total of 69 articles were identified; we included three studies with 1550 participants. For the three HPV16/18 E6 and E7 antibody tests, enzyme-linked immunosorbent assay-based assays had a sensitivity of 18% for detecting CIN2+

†Died February 26, 2022.

(95% confidence interval [CI]: 15–21) and a specificity of 96% (95% CI: 92–98), for slot-blot, sensitivity was 28.9% (95% CI: 23.3–35.1) and specificity was 72% (95% CI: 66.6–77.0) for detecting CIN2+, and for multiplex HPV serology assay based on a glutathione S-transferase, sensitivity was 16% (95% CI: 8.45–28.6) and specificity was 98% (95% CI: 97–99) for detecting invasive cervical cancer. HR-HPV16/18 E6 and E7 serological markers showed high specificity, but sensitivity was suboptimal for the detection of cervical cancer in either population screening settings or as point-of-care screening tests.

KEYWORDS
cervical cancer, E6 and E7 proteins antibody, human papillomavirus, sensitivity, specificity

1 | INTRODUCTION

In high-income countries, organized and effective screening of cervical cancer using Papanicolaou (pap) smears and more recently human papillomavirus (HPV) DNA testing (self-collected vaginal sample) have contributed to a decline in incident cervical cancer cases.[1] However, in low- and middle-income countries (LMICs), despite efforts to implement organized cervical cancer screening with cytology or HPV testing, coverage and uptake rates are very low, with only about 30% of women having undergone opportunistic cervical cancer screening.[2–5] The great benefits and small harm of pap screening have been well documented.[6,7] However, some sociocultural barriers (i.e., health-seeking behaviors, an embarrassment for pelvic examination by male providers, a perception that the pap smear screening process is painful and can include bleeding with a long turnaround time) exist around the genital examination, as shown in a recent review.[8] Therefore, conventional methods for screening cervical cancer that involve genital examination could be considered less desirable or acceptable to women. These include the more current HPV DNA or messenger ribonucleic acid (mRNA) tests that are favored over ThinPrep cytological test and cytology (Pap tests).[9]

In all the aforementioned screening test scenarios, large proportions of women who screen positive for being at high risk of developing cervical cancer require recall and confirmatory diagnostic testing, and then an additional recall to receive treatment. Visual inspection of the cervix with acetic acid (VIA) is an alternative cervical cancer "screen and treat" screening method that is deemed more viable in LMIC, thus avoiding one recall step.[10] However, studies in LMIC have shown mixed results on the performance of VIA in these settings.[7,10,11] Besides, VIA has a problem of high false-positive and false-negative results and depends on the operator's experience.[12,13] As a result, the incidence rates of cervical cancer remain high in LMIC,[5] and reducing incidence rates of cervical cancers in these settings remains a challenge. Hence, a novel efficient screening tool that is less invasive and more acceptable to women is needed.

In May 2018, the General Director of the World Health Organization (WHO) made a global call for action to eliminate cervical cancer using novel technologies for the identification of premalignant tumors at the earliest stage.[14,15] The 90–70–90 global cervical cancer elimination strategies call for 90% of girls to be fully vaccinated by the HPV vaccine by the age of 15 years, 70% of women should have been screened with high-throughput tests by the age of 35 years and again by the age of 45 years, and 90% of women diagnosed with cervical cancer disease to be on treatment by the year 2030.[14]

One of the promising strategies is the use of serum profiling antibodies to HPV16 and HPV18 early oncoproteins E6 and E7 as biomarkers for early detection of invasive cervical cancer (ICC).[16] The E6 protein binds to the E3 ubiquitin ligase E6-associated protein and to the tumor suppressor protein p53, which is involved in the control of the cell growth process and apoptosis.[17] The presence of E7 in human cells prevents phosphorylation of the retinoblastoma protein leading to uncontrolled cell proliferation.[18] Out of the seven early proteins, both E6/E7 oncoproteins are involved in the transformation of cells, mitosis, and immortalization of cervical cancer cells,[19] and have shown the strongest associations with cervical cancer.[20–23] Since several antibody-based finger prick "point-of-care" tests are now readily available for screening other infectious agents such as HIV,[24] which have a short turnaround time (<1 h), a yet-to-be-developed rapid antibody-based HPV finger prick screening test for detecting cervical cancer may be more acceptable than conventional cervical cancer screening methodologies requiring a genital examination. If such a serological test can be carried out cheaply and has a rapid turnaround time, then it could save time and avoid repeat visits.[25]

However, the value of high-risk HPV (HR-HPV) E6 and E7 serology as an alternative, less-invasive point-of-care screening tool for detecting at-risk individuals has not been fully investigated. In addition, globally there are no currently developed and USA Food and Drug Administration (FDA)-approved serological markers for screening cervical cancer in the general population. Studies from the early 1990s[26,27] showed some potential with sensitivities around 27%–41% and specificities around 99%–100%. We, therefore, performed a systematic review aimed at assessing the utility of HPV antibodies, especially of HR-HPV16/18 E6 and E7 serological

markers, in the detection of cervical cancer and cervical intraepithelial neoplasia 2/3 (CIN2/3).

2 | METHODS

This systematic review and meta-analysis were registered and published with PROSPERO (CRD42020206036). In the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA),[28] guidelines were followed to evaluate the usefulness of HPV16/18 E6 and E7 serological markers in the detection of CIN2/3 and cervical cancer.

2.1 | Search strategies

The search for studies was done in the following electronic databases from the period 2010 to 2020: PubMed and Embase, using the following search words "HPV," "E6 and E7 protein," "CIN2," CIN3," and "cervical cancer". We did not include high-grade squamous intraepithelial lesion (HSIL) in the search terms because HSIL is based on cytology, while CIN2/3 are based on histology, which incorporates HSIL. After title and abstract review, where possible full copies of the text of appropriate published articles were obtained and assessed for inclusion. Additional searches were done on the references of the initial studies. Our review focused on HPV16 and HPV18 because they are the most common serotypes and are found in 72% of cervical cancer.[29]

2.2 | Criteria for study selection

The study selection involved exporting all identified studies (69) combined in Mendeley into Covidence (https://www.covidence.org/). Covidence is a useful web tool for screening and selecting studies for conducting systematic reviews. For accuracy and consistency, the two independent reviewers (M. G. S. and T. R.) screened the full texts. Disagreements were resolved through discussion; any unresolved disagreements were resolved by the third reviewer (M. M.).

2.3 | Inclusion

Studies were selected if they were in English, cross-sectional, retrospective case–control, or prospective cohort (or nested case–control). Cases were defined as ICC or CIN 2/3. We used author-defined definitions of controls, which broadly used similarly aged women without HPV-related conditions. Diagnostic accuracy-test studies in which the HPV16/18 E6 and E7 serological markers were compared to a cytopathology or histopathology reference standard were included.

2.4 | Exclusion

Studies with a sample size of less than 200 that could overestimate the effect size[30] (of the small studies excluded some used an HPV E6/E7 mRNA marker and the others used a urine Trovagene HPV test), conference papers, abstracts only, and reviews were excluded. Also, studies that assessed the presence of HPV types in ICC or CIN2/3 were excluded. Given the regular improvements in serological techniques, studies were excluded if they were published before the year 2010. We excluded only laboratory-based studies (which did not include controls) and those studies that focused on CIN1 (wrong outcome). Any studies presenting HPV16/18 E6 and E7 DNA within samples of the cervical biopsy were not included because we aimed to assess the utility of anti-HPV antibodies, especially of HR-HPV16/18 E6 and E7 in the serum of patients with ICC or CIN2/3. We further excluded papers that were not on serological testing, that is, the Onco E6 test because it detects the protein itself, whereas the HPV E6/E7 mRNA tests detect the RNA encoding for the oncoproteins. Our study focused on the test accuracy of HPVE6 and E7 oncoprotein antibodies.

2.5 | Participants

The population of interest were women with cervical cancer and CIN2/3. Studies were included if they reported women with cytologically/histologically confirmed cervical cancer or high-risk lesions (e.g., CIN2 and CIN3) in comparison to women with the normal cervical tissue.

2.6 | Index test

The index test was HPV16/18 E6 and E7 and E4 protein serological markers from the serum of the participants. Determination of the threshold for positive or negative results was based on test cut-off values proposed by the manufacturer or the study authors based on validated cut-off points.

2.7 | Reference standard

The reference test was histopathologic confirmation of cervical cancer or CIN2+ in paraffin-embedded tissue sections or cytological confirmation of CIN2/3 or cervical cancer. Methods of diagnosis were noted.

2.8 | Study outcome

The outcome of the study was sensitivity, specificity, and diagnostic odds ratios (DORs) for CIN2/3 and cervical cancer.

2.9 | Data extraction

The two reviewers independently extracted data (M. G. S. and T. R.) for all the papers meeting the inclusion and exclusion criteria. The following information was extracted from the eligible studies: author; year of publication; study location; sample size; the number of cases and controls, cut-offs, and test manufacturer. We extracted percentages of HPV16/18 E6 and E7 (antibodies seropositive) from precancers and cervical cancer cases and controls according to predefined cut-off values. We then extracted frequencies for true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN) for HPV16 (E6 and E7) or HPV18 (E6 and E7) antibodies. In some of the studies, more than one HPV-type antigen was assessed on the same individuals participating in the study. In such cases, each serological biomarker was considered an independent study when extracting the data.

2.10 | Study quality assessment

Assessment of the quality of the studies was done using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.[31] Quality assessments of the studies were based on: a selection of participants' characteristics; the number of included participants with cervical cancer or CIN2+; description of the index test (HPV16/18 E6 and E7) serological markers and the reference test (histology or cytology). The studies were grouped into high quality, low quality, and unclear. Any disagreements were resolved through discussion with the third reviewer (M. M.).

2.11 | Statistical analyses

To achieve our objective, we performed a descriptive analysis of different serological markers by calculating the sensitivity and specificity, using the TP, FP, TN, and FN results and their 95% confidence intervals (CIs). To quantify pooled effects (Supporting Information: Figure S2), random-effects models for meta-analysis were performed. Cochrane's Q-statistic test and the I^2 tests were used to examine heterogeneity.

Second, we assessed the DOR for HPV16/18 E6 and E7 and other seropositive E oncoprotein (E4) antibodies and for CIN2/3 and cervical cancer from the different studies. Other studies have suggested that E4 plays an important role in the detection of cervical cancer when used in combination with other markers.[32] We pooled the sensitivity and specificity for HPV16/18 E6 and E7 serological markers to detect CIN2/3 and cervical cancer. Analysis by different serological screening tests (enzyme-linked immunosorbent assay [ELISA], multiplex HPV serology assay based on a glutathione S-transferase capture immunoassay in combination with fluorescent beads [multiplex HPV serology immunofluorescent assay] and immunoenzymatic assay [slot-blot]) was performed for comparison of pooled effects and heterogeneity. In the primary studies, cases and controls were matched by age.

To estimate sensitivity and specificity, a bivariate random-effects model[33] was applied using "Metandi" and "Midas" in STATA. Metandi fits a two-level mixed logistic regression model, with independent binomial distributions for the TP and TN conditional on the sensitivity and specificity in an individual study, and a bivariate hierarchical normal model for the logit transformation of sensitivity and specificity between studies. Midas calculates summary receiver-operating characteristics (SROC) (Supporting Information: Figure S3), summary likelihood, and DOR. Hierarchical SROC (HSROC) was used to account for the correlation between sensitivity and specificity (Supporting Information: Figure S4). The HSROC accounts for threshold effects and between- and within-study variability.

Statistical significance was considered at a p value of 0.05, and all analyses were performed using STATA version 16 and the Review Manager (Version 5.3; Cochrane Collaboration). All statistical tests were two-sided.

3 | RESULTS

We first identified 69 records (papers) through PubMed library searches and references (Figure 1). We retrieved 44 potential papers after a screening of titles and abstracts. Twenty-three papers were eligible for inclusion in the systematic review. Out of 23 papers, only 3 papers were included with 1550 women. A total of 11 HPV16/18 oncoprotein antibodies (studies) were obtained from different serological test methods. Two-thirds of the studies were a case–control design and one study was a prospective diagnostic accuracy study (Table 1). Assessment of the methodological quality of the individual studies was done using QUADAS-2 (Supporting Information: Figure S1 and Table 2).

There was a considerable degree of heterogeneity for sensitivity between the studies for combined CIN2/3 and ICC, which fluctuated between 0 and 0.40, while specificity was very good for most of the biomarkers. Thus, we did not pool the studies (Figure 2A–C). For the stratified analysis by tumor stage, sensitivity was very low for CIN2/3, which ranged between 0 and 0.20 for the biomarkers, while specificity was above 0.90 for most of the biomarkers. For ICC, the sensitivity ranged between 0.20 and 0.40 and specificity ranged between 0.96 and 0.97.

Different assays were used to characterize the association between HPV E6 and E7 antibodies and cervical cancer. In the studies that used ELISA, the pooled sensitivity was 18% (95% CI: 15–21) and specificity was 96% (95% CI: 92–98). For the studies that used multiplex HPV serology assay, the pooled sensitivity was 16% (95% CI: 8.45–28.6) and specificity was 98% (95% CI: 97–99) (Table 3). For the studies that used immunoenzymatic assay (slot-blot), the pooled sensitivity was 28.9% (95% CI: 23.3–35.1) and specificity was 72% (95% CI: 66.6–77.0) (Table 3). The multiplex immunofluorescent assay had a DOR of 9.72 (95% CI: 3.95–23.93), the ELISA had a DOR of 5.00 (95% CI: 2–11), and immunoenzymatic assay had a DOR of 1.05 (95% CI: 0.72–1.52) (Table 3).

Across the studies, HPV antibody sensitivity was low, ranging from 4% to 40%, while the specificity was high, ranging from 61%

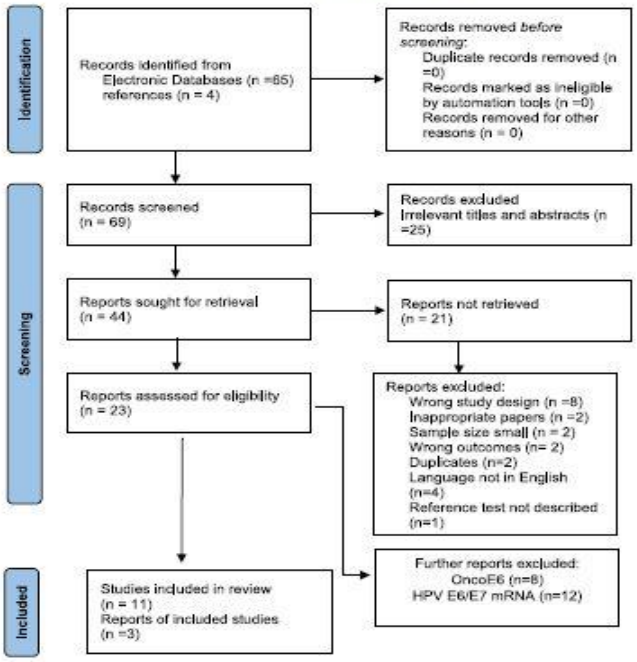


FIGURE 1 The Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram of the studies in the systematic review of HPV16 and HPV18 E6/E7 serological markers. HPV, human papillomavirus.

TABLE 1 Summary of the three study characteristics included in the review: CIN2, CIN3, and ICC

No.	Study ID (author and year)	Study reference number	Country	Study design	Index test	Reference method	Sample size	Median age	CIN2+, *n* (%)	ICC, *n* (%)
1	Salazar-Piña (2016)[20]	d1	Mexico	Diagnostic test accuracy study	HPV16 E4 and E7 immunoenzymatic assay (slot-blot)	Histo-pathology	485	40.0	106 (29.9)	15 (3.1)
2	Jin (2018)[24]	d2	South Korea	Case–control study	HPV E6 and E7 (ELISA)	Cytology	249	44.3	144 (57.8)	-
3	Combes (2014)[25]	d3	Algeria and India	Case–control study	HPV16 L1, E1,E2, E4, E6, and E7 (multiplex HPV serology immunofluorescent assay)	Cytology	816	50.9	-	307 (37.6)

Abbreviations: CIN, cervical intraepithelial neoplasia; ELISA, enzyme-linked immunosorbent assay; HPV, human papillomavirus; ICC, invasive cervical cancer.

to 99%. There was a considerable degree of heterogeneity between the studies; thus, we did not pool the studies (Figure 2). From the HSROC curve, HPV16 E6 antibody presence appears to be a better marker compared to the other markers (Figure 3).

4 | DISCUSSION

Antibodies to HPV oncoproteins have been suggested as important serological markers in the detection of cervical cancer. Our systematic review and meta-analysis focused on the sensitivity and specificity of

TABLE 2 True positive, false positive, false negative, and true negative of different tests for the detection of CIN2/3 and ICC

No.	Study ID (1st author and year)	Study reference number	CIN2, CIN3, or cervical cancer)	Cut-off (threshold)	Test (manufacturer)	Marker name (index test)	Full name	TP	FP	FN	TN
1	Salazar-Piña (2016)[20]	d1a	CIN2+	2AU/ml	Qiagen	HPV16 E7 (slot-blot)	HPV 16 E7 antibodies immunoenzymatic assay (slot-blot)	48	58	73	92
	Salazar-Piña (2016)[20]	d1b	CIN2+	7AU/ml	Qiagen	HPV16 E4 (slot-blot)	HPV16 E4 antibodies immunoenzymatic assay (slot-blot)	22	26	99	124
2	Jin (2018)[14]	d2a	CIN2+	N/A	Greiner Bio-One	HPV16 E6 (ELISA)	Enzyme-linked immunosorbent assay	41	2	118	47
	Jin (2018)[14]	d2b	CIN2+	N/A	Greiner Bio-One	HPV16 E7 (ELISA)	Enzyme-linked immunosorbent assay	28	2	131	47
	Jin (2018)[14]	d2c	CIN2+	N/A	Greiner Bio-One	HPV18 E6 (ELISA)	Enzyme-linked immunosorbent assay	29	2	130	47
	Jin (2018)[14]	d2d	CIN2+	N/A	Greiner Bio-One	HPV18 E7 (ELISA)	Enzyme-linked immunosorbent assay	26	2	133	47
3	Combes (2014)[35]	d3a	ICC	394-714 MFI	Luminex Corp.	HPV16E6 (multiplex)	Multiplex HPV serology immunofluorescent assay	99	4	208	323
	Combes (2014)[35]	d3b	ICC	395-714 MFI	Luminex Corp.	HPV16E7 (multiplex)	Multiplex HPV serology immunofluorescent assay	86	10	221	317
	Combes (2014)[35]	d3c	ICC	396-714 MFI	Luminex Corp.	HPV18E6 (multiplex)	Multiplex HPV serology immunofluorescent assay	13	9	294	318
	Combes (2014)[35]	d3d	ICC	397-714MFI	Luminex Corp.	HPV18E7 (Multiplex)	Multiplex HPV serology immunofluorescent assay	39	5	268	322
	Combes (2014)[35]	d3e	ICC	394-714MFI	Luminex Corp.	HPV16 E4 (multiplex)	Multiplex HPV serology immunofluorescent assay	53	4	254	323

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ICC, invasive cervical cancer; N/A, not available.

FIGURE 2 Forest plots of sensitivity and specificity for different serological marker studies in the detection of CIN2/3 and ICC. (A) Forest plots of sensitivity and specificity for different serological markers studies in the detection of combined CIN2/3 and ICC. (B) Forest plots of sensitivity and specificity for different serological marker studies in the detection of CIN2/3. (C) Forest plots of sensitivity and specificity for different serological marker studies in the detection of ICC. CI, confidence interval; CIN, cervical intraepithelial neoplasia; FN, false negative; FP, false positive; HPV, human papillomavirus; ICC, invasive cervical cancer; TN, true negative; TP, true positive.

HPV16/18 early oncoprotein antibodies. Our main findings suggest that HPV16 and HPV18 E6 and E7 antibodies have high specificity but low sensitivity to detect cases with cervical cancer or CIN2/3 precancerous lesions. Another key finding is that there is scant literature on E6/E7 as a biomarker for ICC and CIN2/3.

We found that HPV16 and HPV18 E6 and E7 antibodies have higher specificity but lower sensitivity. Our estimates are similar to the estimates pre-2010 of Sun et al.[36] in Brazil where they reported specificity of 99.5% and sensitivity of 40.7% for HPV16 E6 or E7 (higher antibody titers). Similarly, in Japan, the findings of Sasagawa et al,[27] using ELISA test with E6 or E7 proteins as antigens, had first shown in 1992 that 27% of cervical cancer and 19% of CIN3 were positive for anti-HPV16 E6 antibody, while 33% in cervical cancer and 8% in CIN3 were positive for anti-HPV16 E7. Therefore, low sensitivity in these serological tests may represent a lower immune response against HPV16/18 E6 or E7 protein in many patients with these malignant lesions. Another explanation for the low sensitivity of these assays might be due to low immune responses against HPV16/18. The serological responses to HPV16/18 E6 and E7 are likely to be surrogate markers for cytotoxic immune responses by cytotoxic T lymphocyte (CTL) targeting these proteins. Such CTLs function to kill CIN or cancer cells positive with HPV16 or HPV18.[37] In addition, our findings showed that the HPV16 E6 antibody is a relatively better serological marker compared to the other biomarkers. Similarly, a recent systematic review and meta-analysis on blood-based biomarkers of HPV-associated cancer found HPV16 E6 antibodies to be a better serology marker.[38] Although HPV16 E6 was shown to be a better biomarker, its sensitivity is still low for it to be used as a screening tool. Thus, additional studies are needed for its validation with other biomarkers.[39]

HPV16 E6 and E7 antibodies have been associated with the tumor stage of cervical cancer.[37,39-42] In our study, we found that the sensitivity for CIN2/3 was lower compared to the sensitivity for ICC. Our findings are in agreement with the findings of Park et al.,[40] who reported that seropositive HPV16 E6 and E7 antibodies were associated with the stage of the tumor. Their findings showed that seropositivity for HPV16 E6 was 51% and 38% for HPV16 E7 in Stage II and HPV16 E6 (69%) and HPV16 E7 (56%) for Stage III/IV, respectively. In addition, in squamous carcinoma seropositivity was 54% for HPV16 E6% and 32% for HPV16 E7. However, a radioimmunoprecipitation assay (RIPA) was used making comparison difficult between assays. Similarly, in Korea, Chee et al.,[37] using RIPA, found that HPV16 E6 and E7 antibodies were associated with the clinical stage of the tumor for HPV16 E6 (CIN [4.2%], Stage I [12.5%], Stage IIa [35.7%], Stage IIb [100%], Stage III [100%]) and HPV16 E7 (CIN [4.2%], Stage I [43.8%], Stage IIa [57.1%], Stage IIb [60%], Stage III [100%]).

The strengths of our systematic review and meta-analysis include the application of the standard systematic review protocol, using Covidence software to screen the studies, involvement of second and third

TABLE 3 Bivariate meta-analysis of different HPV16 and HPV18 serological markers: pooled sensitivity and specificity by assay type

	Marker name	Study reference number	Number of studies	Endpoint	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95% CI)
HPV serology (ELISA)	HPV16 and HPV18 (E6 and E7)	d2a, d2b, d2c, d2d	4	CIN2+	18.0 (15–21)	96.0 (92.0–98.0)	5.00 (2.00–11.00)
HPV serology (multiplex HPV serology immunofluorescent assay)	HPV16 and HPV18 E6, E7, and E4	d3a, d3b, d3c, d3d, d3e	5	ICC	16.0 (8.45–28.6)	98.0 (97.0–99.0)	9.72 (3.95–23.93)
HPV serology (immunoenzymatic assay (slot-blot))	HPV16 E7 and E4	d1a, d1b	2	CIN2+	28.9 (23.3–35.1)	72.0 (66.6–77.0)	1.05 (0.72–1.52)

Abbreviations: CI, confidence interval; DOR, diagnostic odds ratio; ELISA, enzyme-linked immunosorbent assay; HPV, human papillomavirus; ICC, invasive cervical cancer.

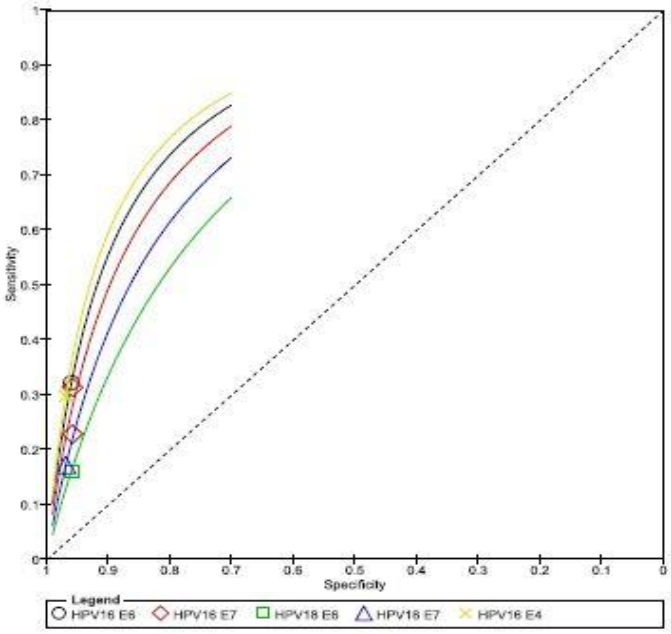


FIGURE 3 Summary of the hierarchial summary receiver-operating characteristic plots of sensitivity and specificity for HPV16 and HPV18 protein serological markers, namely, E6, E7, and E4. HPV, human papillomavirus.

reviewers at all stages of screening, and using QUADAS-2 to assess the quality of the studies' methodology. Our study had several limitations: After all the exclusions, there were only a few studies included that assessed the antibodies against HPV E6 and E7 oncoprotein for detection of CIN2/3 and cervical cancer. There was a high level of heterogeneity for the included studies; therefore, it was impossible to have pooled estimates of all the studies. Due to the different assays used, we were unable to pool all the studies together. In addition, due to the selection bias that comes with case–control design, we were unable to pool the studies together.[43] Our systematic review and meta-analysis only covered studies published in the last 10 years and might have missed studies published in other languages, or that were not appearing during the literature search.

This systematic review and meta-analysis focused on the utility (e.g., sensitivity and specificity) of HPV antibodies, especially of HPV16/18 E6 and E7 serological markers in the detection of ICC and CIN2/3. Recently, the WHO in their new cervical cancer guidelines suggested HPV antibodies and onco-proteins as possible future screening tests.[44] If such a test could be incorporated into population screening using point-of-care

testing of appropriately aged nonvaccinated women (e.g., 30–65 years as recommended by cervical screening guidelines),[44] it would improve screening rates, reduce losses to follow-up (people, samples, and results) and ultimately lead to early detection of the disease in limited-resource settings. Individuals who have been seropositive to both HPV E6/E7 antibodies are at higher risk for cervical cancer,[45] which makes them potentially useful biomarker candidates for early detection of cervical cancer.[46] However, we found that evidence is still limited for HPV antibodies to be translated into an effective detection tool for cervical cancer detection. Further laboratory studies are required on the development of more accurate HPV serological biomarkers for them to be successfully translated into an effective screening or point-of-care detection tool for cervical cancer.

Our systematic review and meta-analysis further add to the debate that HPV16 and 18 E6 and E7 antibodies could be useful biomarkers for the screening of cervical cancer. There seems very little improvement over time in the utility of serological markers in detecting cervical cancer or CIN3. Although the specificity of HPV serological markers is high, their sensitivity is suboptimal for the detection of cervical cancer.

AUTHOR CONTRIBUTIONS

Mwiza Gideon Singini conceptualized the study, performed data analysis, and drafted the manuscript. Freddy Sitas, Tim Waterboer, Chantal Babb de Villiers, and Elvira Singh edited the manuscript. Melitah Motlhale and Thendo Ramaliba assisted with data extraction. Mazvita Muchengeti, Abram Bunya Kamiza, and Robert Newton assisted in proofreading the manuscript. Wenlong Carl Chen helped with the formatting of figures. Debbie Bradshaw, Tim Waterboer, Christopher G. Mathew, Wenlong Carl Chen, Freddy Sitas, Elvira Singh, Mazvita Muchengeti, Abram Bunya Kamiza, and Robert Newton are the members of the ERICA-SA collaborative study. All authors read and provided feedback to improve the final version of the manuscript.

ACKNOWLEDGMENTS

We thank all those who provided feedback to improve the manuscript. This study was supported by the South African Medical Research Council (with funds received from the South African National Department of Health) and the UK Medical Research Council (with funds from the UK Government's Newton Fund) (MRC-RFA-SHIP 01-2015). The funders were not involved in the conceptualization, review, or approval of the manuscript. This project forms part of an international research program aimed at identifying evolving risk factors for cancer in African populations (ERICA-SA) (https://www.samrc.ac.za/intramural-research-units/evolving-risk-factors-cancers-african-populations-erica-sa).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Mwiza Gideon Singini http://orcid.org/0000-0002-2897-1728
Elvira Singh http://orcid.org/0000-0003-1259-2122
Debbie Bradshaw http://orcid.org/0000-0002-3588-2184
Wenlong Carl Chen http://orcid.org/0000-0002-3248-4906
Melitah Motlhale http://orcid.org/0000-0003-4703-7057
Abram Bunya Kamiza http://orcid.org/0000-0002-3919-7651
Chantal Babb de Villiers http://orcid.org/0000-0003-1334-1819
Mazvita Muchengeti http://orcid.org/0000-0002-1955-923X
Robert Newton http://orcid.org/0000-0001-6715-9153
Noemi Bender http://orcid.org/0000-0003-2542-9949
Freddy Sitas http://orcid.org/0000-0001-9679-1481

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Singini MG, Singh E, Bradshaw D, et al. Usefulness of high-risk HPV early oncoprotein (E6 and E7) serological markers in the detection of cervical cancer: a systematic review and meta-analysis. *J Med Virol*. 2022;1-10. doi:10.1002/jmv.27900

Singini et al. Infectious Agents and Cancer (2022) 17:14
https://doi.org/10.1186/s13027-022-00418-2

Infectious Agents and Cancer

RESEARCH ARTICLE Open Access

HPV types 16/18 L1 E6 and E7 proteins seropositivity and cervical cancer risk in HIV-positive and HIV-negative black South African women

Mwiza Gideon Singini[1,2], Elvira Singh[1,2*], Debbie Bradshaw[3], Wenlong Carl Chen[1,4], Melitah Motlhale[1,2], Abram Bunya Kamiza[4], Chantal Babb de Villiers[5], Mazvita Muchengeti[1,2,9], Christopher G. Mathew[4,6], Robert Newton[7,8], Noemi Bender[10], Tim Waterboer[10] and Freddy Sitas[3,11,12]

Abstract

Background: In populations with high rates of human immunodeficiency virus (HIV)-coinfection, the nature of the relationship between human papillomavirus (HPV)-16 and -18 (L1, E6 and E7) antibodies and cervical cancer is still uncertain. We measured the association between seropositivity to HPV (L1, E6 and E7) proteins and cervical cancer among black South African women with and without HIV co-infection.

Methods: We used questionnaire data and serum collected from consecutively recruited patients with a newly diagnosed cancer from the Johannesburg Cancer Study from 1346 cervical cancer cases and 2532 controls (diagnosed with other non-infection related cancers). Seropositivity to HPV proteins was measured using a multiplex serological assay based on recombinant glutathione S-transferase (GST) fusion proteins. We measured associations between their presence and cervical cancer using unconditional logistic regression models and evaluated the sensitivity and specificity of these HPV biomarkers.

Results: Among controls, HIV-negative women from rural areas compared to urban had significantly higher HPV seroprevalence, HPV16 E7 (8.6% vs 3.7%) and HPV18 E7 (7.9% vs 2.0%). HPV16 E6 and E7 antibodies were positively associated with cervical cancer in HIV-positive (Adjusted Odds Ratio (AOR) = 33; 95% CI 10–107) and HIV-negative women (AOR = 97; 95% CI 46–203). In HIV-positive women, HPV E6/E7 antibodies had low sensitivity (43.0%) and high specificity (90.6%) for cervical cancer detection. In HIV-negative women, HPV E6/E7 antibodies sensitivity was 70.6% and specificity was 89.7%.

Conclusions: Our data show that HPV (L1, especially E6 and E7) antibody positivity is associated with cervical cancer in both HIV-positive and HIV-negative women. Nonetheless, being HIV-positive plays an important role in the development of cervical cancer.

Keywords: Cervical cancer, Human papillomavirus, E6 and E7, L1 proteins, Seropositivity, South Africa

Introduction

Globally, in 2018, about 70% of cervical cancers were attributable to high-risk human papillomavirus (HPV) types 16 and 18 [1]. In South Africa, HPV16 and 18 are

*Correspondence: elviras@nicd.ac.za
[1] National Cancer Registry, National Health Laboratory Service, Sandringham, Johannesburg, South Africa
Full list of author information is available at the end of the article

important causes of cervical cancer and are included in the currently available HPV vaccine.

The HPV early (E6 and E7) oncoproteins and the late (L1) protein are encoded by the HPV genome. The HPV oncoproteins play an important role in the tumorigenesis of cervical cancer [2]. Humoral immune response against major capsid late protein (L1) is generated during infection with HPV; which is a marker of past or present infection [3]. Furthermore, integration of the HPV genome in the host cell results in overexpression of E6 and E7 oncoproteins, which are involved in the transformation and progression of cervical and other HPV-related cancers [4]. Different serological assays have been used to screen for HPV-16 and -18 (E6 and E7) [5–8] oncoprotein antibodies. Serological markers for HPV-antibodies provide useful data about past and current HPV infections and their relationship to cervical cancer [9, 10].

Previous studies have shown that HPV types 16 and 18 L1, E6 and E7 antibody proteins are associated with cervical cancer susceptibility [6, 8, 11–13] however, with varying degrees of strength of association. For example, in a case–control study from Russia, Zumbach et al. [8] utilized an enzyme-linked immunosorbent assay (ELISA) and reported odds ratios (ORs) for cervical cancer of 64.4 (95% CI 3.8–1085) for HPV16 E6 and 4.9 (95% CI 1.3–18.7) for HPV16 E7. In another case–control study from Algeria and India, Combes et al. [10] used a multiplex immunofluorescent HPV serology assay and reported ORs for cervical cancer of 37.1 (95% CI 13.4–103) for HPV16 E6, 12.5 (95% CI 6.3–24.8) for HPV16 E7 and 5.9 (95% CI 3.1–11.1) for HPV16 L1. Earlier work on the Johannesburg Cancer Study (JCS) from 946 human immunodeficiency virus (HIV)-negative women with cervical cancer and 1,342 controls using an ELISA assay and different cut-offs found ORs between moderate and high HPV-16L1 seropositivity of 1.5 (95% CI 1.2–1.9) and 2.4 (95% CI 1.9–3.0) [14]. Thus, multiple serologic methods had been used to characterize the relationship between cervical cancer incidence and HPV.

Less is known about the role of high-risk HPV oncoproteins among cervical cancer patients in black African women who are HIV-positive and HIV-negative. In South Africa, despite high HIV prevalence (24.1%) among black South African women [15], studies on HPV16 and 18 (E6 and E7) and late (L1) seropositivity and the risk of cervical cancer are still lacking. We therefore assessed the seropositivity of HPV -16 and -18 early (E6 and E7), and late (L1) antibodies among cervical cancer cases and infection unrelated cancer controls using a multiplex HPV serology assay [16], and estimated their association with cervical cancer risk among HIV-positive and HIV-negative women.

Methods

Setting and participants

The study population were participants recruited into the Johannesburg Cancer Study (JCS) between 1995 and 2016. The aims of JCS included measuring the relative importance of known and emerging risk factors for cancer in a black African population in Johannesburg, South Africa. The details of the JCS have been described elsewhere [17]. Briefly, the JCS collected serum samples from 26,000 consecutive consenting black South African patients with newly diagnosed cancers (>90% histopathology confirmed), referred to the medical oncology and radiation therapy wards of (the main tertiary public) Charlotte Maxeke Johannesburg Academic Hospital and associated referral hospitals and clinics. Self-reported data on demographics and key lifestyle risk factors were collected using a structured questionnaire. The JCS also collected venous blood in one serum separation tube (SST) and one Ethylenediaminetetraacetic acid (EDTA) tube. SST serum was separated from whole blood within two days of sample collection and the sample was divided into a maximum of 4 aliquots. HIV testing was done using the Vironostika HIV Ag/Ab kit [18]. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number: M200252).

Cases and controls

The JCS is amenable to a case–control design and analysis by selecting controls that are unrelated to the exposures of interest. The current study was restricted to women aged 25 to 54 years because a large proportion of the older women did not have serology samples available. Cases were 1346 women with newly diagnosed cervical cancer (C53). Controls were 2532 women with newly diagnosed infection unrelated cancers (breast (C50) (n = 1953), colon (C18-20) (n = 197), oesophagus (C15) (n = 48), endometrium (C54-55) (n = 75), lung (C33-34) (n = 76), pancreas (C25) (n = 21) and minor cancer types (n = 162) (Additional file 1: Table S1)). Figure 1 outlines the criteria used to select cases and controls.

Serology data and laboratory methods

Serum aliquots were stored at − 25 °C and one aliquot was shipped on dry ice to the German Cancer Research Center (Deutsches Krebsforschungszentrum (DKFZ)) in Heidelberg, for antibody testing for HPV16 and 18 (L1, E6 and E7) using a multiplex serological assay. This was based on a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads on a Luminex platform [16]. The final net (bead and GST background subtracted) Median Fluorescence Intensity (MFI) values of all samples were analysed at a dilution of 1:1000. Previously established standard

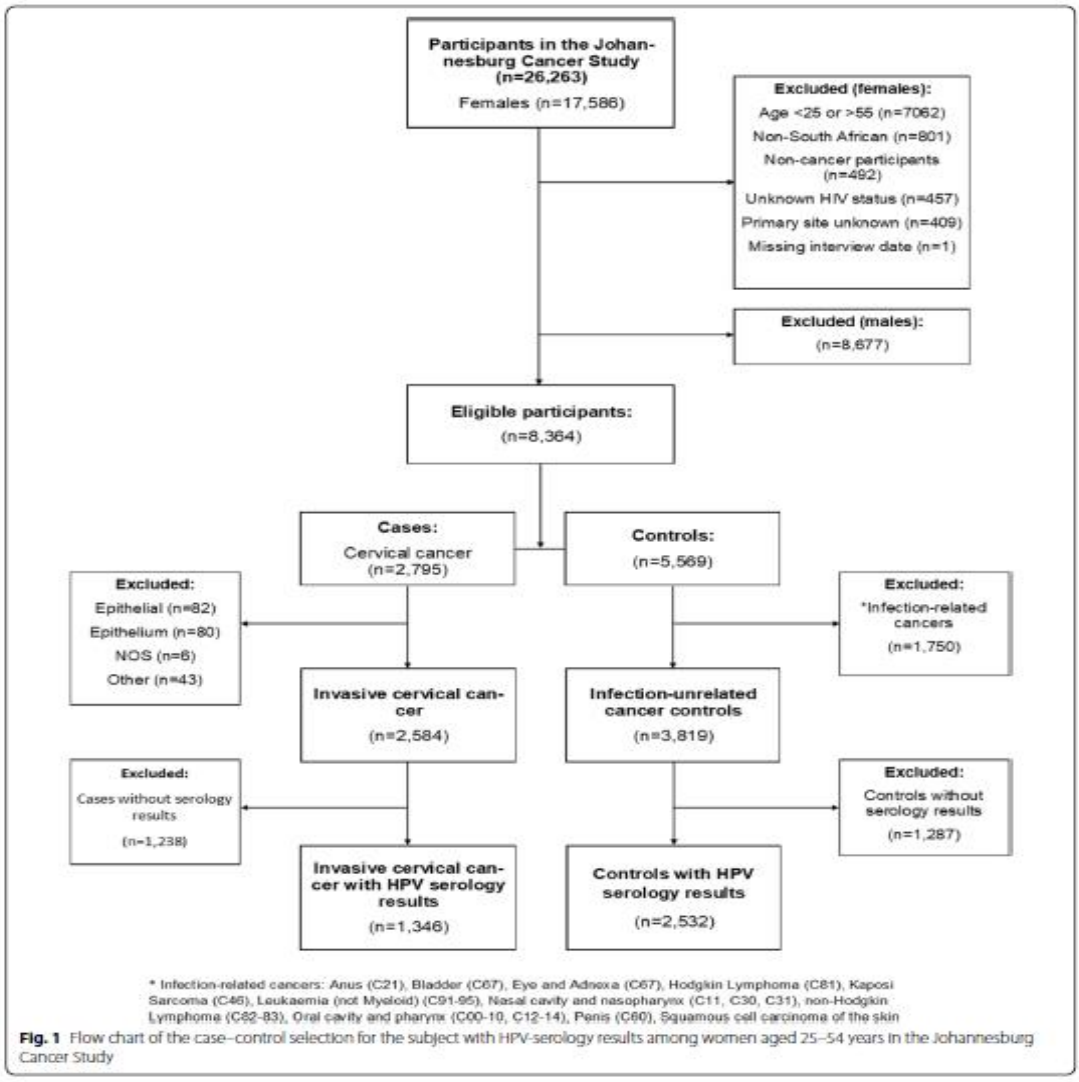

Fig. 1 Flow chart of the case–control selection for the subject with HPV-serology results among women aged 25–54 years in the Johannesburg Cancer Study

cut-offs were generated at 1:100 serum dilution [19, 20] and were thus not applicable. Using the Visual Inflection Point (VIP) method [21], we defined net MFI cut-offs of 175 for HPV16 and 18 L1, 75 for HPV16 and 18 E6, 120 for HPV16 E7, and 70 for HPV18 E7. For HPV antibodies, we generated five extra binary variables to describe high-risk combinations for the presence of either E6 /E7 singly or in combination (HPV16 E6 & E7, HPV18 E6 & E7, HPV16&18 E6, HPV16 &18 E7, and HPV16/18 E6/E7).

Statistical analysis

Data on demographic characteristics were summarized using frequencies and percentages for the categorical variables, medians and the interquartile range (IQR) for the continuous variable that was not normally distributed. A Pearson's Chi-squared test (for categorical variables) and t-test (for age) were used for the comparison of demographics between cases and controls. To assess cross-reactivity between the proteins, a tetrachoric correlation coefficient rank test [22] with Bonferroni adjustments (to take account of multiple comparisons) was used to analyze the correlation between each HPV16 and 18 (L1, E6 and E7) antibodies and HIV antibodies (See Additional file 1: Fig. S2). In the control group, we calculated the seroprevalence of antibodies to HPV16 and 18 (E6 and E7) oncoproteins and L1 protein across different demographic and lifestyle factors, such as age (25–34, 35–44, 45–54 years), place of residence (rural, urban), period of interview (1995–1999, 2000–2004, 2005–2009, 2010–2016), marital status (never married, married, previously married), educational level (none, primary, secondary and above), number of sexual partners (0–1, 2–5, 6+) HIV-status (negative and positive) and parity (0, 1, 2, 3, 4+). We have previously shown that cancer types unrelated to the exposure of interest, resemble background population prevalence for that exposure [18, 23]. We performed a test for heterogeneity on categorical variables and a score test for trends in proportions for ordinal categorical variables. On the assumption that the selected controls should have similar seroprevalences to each other, we compared heterogeneity in seroprevalence of HPV16 and 18 (L1, E6 and E7) antibodies across different control cancer types using the Cochran-Mantel–Haenszel test (See Additional file 1: Fig. S1).

We used an unconditional logistic regression model to assess the association by calculating adjusted Odds Ratios (AOR) and 95% confidence intervals between HPV16 and 18 (E6 and E7) and L1 proteins seropositivity and the risk of cervical cancer. We stratified by HIV status to assess if the seroprevalence of the HPV proteins differs in HIV-positive and HIV- negative patients. We adjusted for age, education level, number of sexual partners, marital status, period of interview and place of residence. To assess whether the clinical performance of HPV-16 and -18 antibodies as a diagnostic marker for cervical cancer we performed the same in HIV-positive and HIV-negative cancer patients. We calculated the sensitivity, specificity of the various HPV antibodies for detecting cervical cancer using the "diagti" command in STATA. In addition, the area under the ROC curve (AUC) of the receiver operator characteristics was computed to compare the performance of the best combination of HPV E6

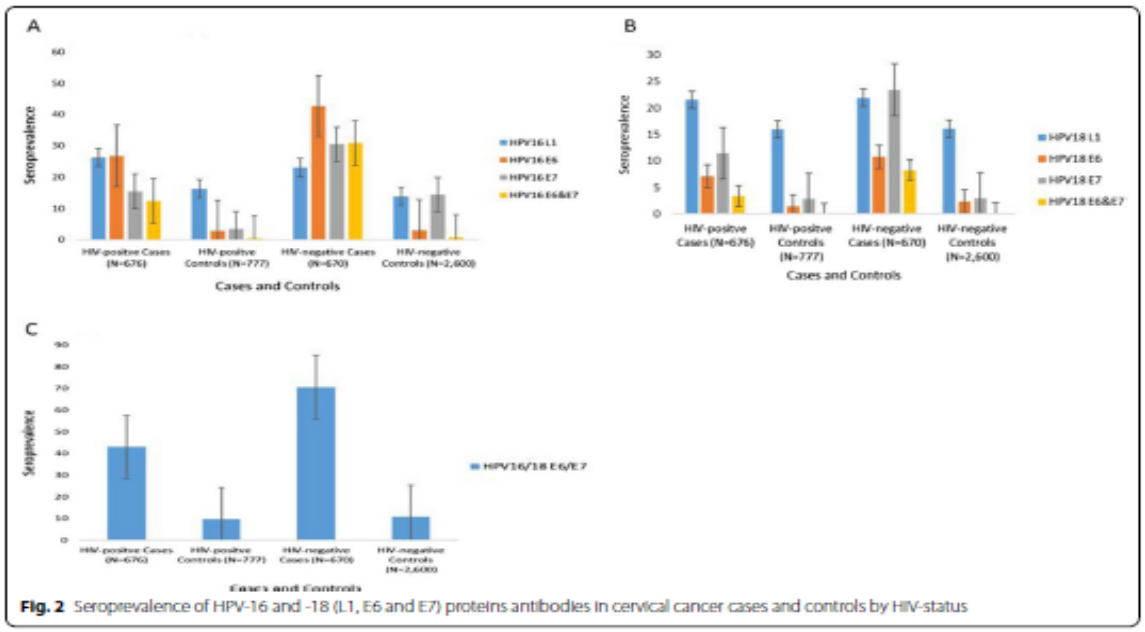

Fig. 2 Seroprevalence of HPV-16 and -18 (L1, E6 and E7) proteins antibodies in cervical cancer cases and controls by HIV-status

and E7 antibodies to discriminate cervical cancer from controls (See Additional file 1: Table S4). All the statistical tests were computed using STATA software version 16.0 (Stata Corp, college station, Tx) and SAS v 9.4 (SAS Institute, Cary, NC). All tests were considered significant at a two-tailed alpha of 5%.

Results

Out of a total of 2,795 cervical cancer cases and 5,569 infection unrelated cancer controls participating in the JCS, 3,878 women (1,346 cases and 2,532 controls) aged 25–54 years were included (Fig. 1). Cases were relatively younger (Median age: 42 (IQR: 37–46) compared to controls (Median age: 44 (IQR: 39–50) (p value < 0.001). At least two-thirds of both cases (63.5%) and controls (72.4%) had a secondary school education (Table 1).

Overall, 50.2% of women with cervical cancer were HIV positive vs. 26.3% HIV positive controls (Table 1). Furthermore, 13.5% of HPV16 L1 seropositive women were also HPV18 L1 seropositive (Additional file 1: Table S3).

Stratifying by HIV-status, HPV16 E6 & E7 and HPV18 E6 & E7 seropositivities were higher among HIV-negative women amongst both cases HPV16 E6 (42.7%) and controls HPV16 E6 (3.0%) as compared to HIV-positive women cases HPV16 E6 (26.8%) and controls HPV16 E6 (2.8%) (Fig. 2).

Among HIV-negative controls, the overall antibody seroprevalence for HPV16 L1 was 14.3% and 0.5% for combined HPV16 E6&E7. Similar observations were made for HPV18 L1 (16.3%) and for combined HPV18 E6&E7 (0.2%) (Table 2). In HIV-positive controls, the overall antibody seroprevalence was 15.9% for HPV16 L1, HPV18 L1(15.7%), 0.5% for HPV16 E6 and E7 and 0.2% for HPV 18 E6 and E7 (Table 3).

In general, we did not observe differences in seroprevalence to HPV types 16 and 18 L1 and (E6 and E7) antibodies by demographic and sexual/reproductive factors (p value > 0.05). Among HIV-negative controls, the prevalence of HPV16 E7 antibody was higher among women that lived in rural areas (8.6%) as compared to women who lived in urban areas (3.7%) (p value for heterogeneity = 0.005) (Table 2). A similar pattern was observed for HPV18 E7 (rural (7.9%) vs urban (2.0%); p value = 0.001). Among HIV-positive controls, the prevalence of HPV16 E6 &E7 antibodies decreased with an increase in number births up to 2 births (p value = 0.006) (Table 3).

Being seropositive to HPV16 antibodies was significantly associated with cervical cancer: combined HPV16 E6 and 7 (AOR = 69.20, 95% CI 37.07–129.18), HPV16 E6 (AOR = 21.96, 95% CI 16.61–29.02), HPV16 E7 (AOR = 7.93, 95% CI 6.16–10.22) and HPV16 L1 (AOR = 1.74, 95% CI 1.45–2.07). For HPV18, antibody seroprevalence ORs for cervical cancer ranged from AOR = 38.61 (95% CI 15.13–98.53) for combined HPV18 E6&E7, AOR = 8.94 (95% CI 6.64–12.03) for HPV18 E7, AOR = 4.67 (95% CI 3.30–6.50) for HPV18 E6 and AOR = 1.31 (95% CI 1.10–1.56) for HPV18 L1 (Table 4).

In general, HPV L1 cervical cancer ORs were higher in HIV-positive women but lower in HIV-negative women. The AORs of cervical cancer for combined HPV16 E6 & E7 seropositivity were 97.40 (95% CI 46.68–203.23) in HIV-negative, and 33.10 (95% CI 10.22–107.20) in HIV-positive women (Fig. 3).

Among HIV-positive women, HPV16/18 E6/E7 antibodies had a sensitivity of 43.0%, specificity of 90.6% and AUC of 67% to detect cervical cancer. Among HIV-negative women, the sensitivity, specificity and AUC for HPV16/18 E6/E7 antibodies was 70.6%, 89.7% and 80% respectively for detection of cervical cancer. The sensitivity, specificity and AUC for discriminating cervical cancer based on HPV16 E6 positivity was 26.8%, 97.2% and 62% respectively in HIV-positive women. For HIV-negative women who were HPV E6 seropositive, the sensitivity was 42.7%, specificity was 95.6% and the AUC 69% (Table 5).

Discussion

In this study, we assessed antibody seropositivity to each of HPV-16 and -18 oncoproteins (E6 and E7), and L1 protein and calculated their association with cervical cancer. While the seroprevalence of HPV16 L1 in the previous JCS study among HIV-seronegative women was higher [14] than in this study, ORs between HPV16 L1 and cervical cancer were about the same (overall crude OR 2.2; 95% CI 1.8–2.6, vs 2.1; 1.5–2.7, Fig. 3). Previous data on HPV16 L1 seroprevalence in controls reflect different assay methods and cutoffs used, and perhaps some cross-reactivity with other HPV types. Our key findings, using larger sample size, more recent serological methods measuring a range of high-risk HPV types showed the important role of HPV-16 and -18 (E6 and E7) oncoproteins in cervical cancer risk in both HIV-positive and HIV-negative cancer patients. HPV-16 and -18 (E6 and E7) oncoproteins were associated in cervical cancer risk in both HIV-positive and HIV-negative cancer patients. HIV-positive patients compared to HIV-negative patients had lower sensitivity but with both high specificity for HPV16 and HPV 18(E6 and E7). Findings from our study confirmed that, in HIV-negative controls, HPV16 E7 and HPV18 E7 seroprevalence differed by place of residence. Among HIV-positive women, HPV16 E6&E7 seroprevalence increased with an increase in number of births.

The higher seroprevalence of HPV16 E6, HPV16 E7 and HPV18 E7 in women from rural areas might indicate a greater number of women with early undetected cervical or related cancer lesions. In rural areas of

Table 1 Comparison of demographic characteristics of study participants from the JCS (1995 – 2016)

Characteristics	Total (N = 3878) N (%)	Cervical cancer Cases (N = 1346) n (%)	Controls (N = 2532) n (%)	Comparison of cases and controls *p* value
Age				
Median (IQR)	44 (38–48)	42 (37–46)	44 (39–50)	**< 0.001**[#]
25–34	528 (13.6)	186 (13.8)	342 (13.5)	**< 0.001**[$]
35–44	1,631 (42.1)	693 (51.5)	938 (37.1)	
45–54	1,719 (44.3)	467 (34.7)	1,252 (49.5)	
Period of Interview				
1995–1999	255 (6.6)	133 (9.9)	122 (4.8)	**< 0.001**
2000–2004	717 (18.5)	254 (18.9)	463 (18.3)	
2005–2009	1,431 (36.9)	538 (40.0)	893 (35.3)	
2010–2016	1,475 (38.0)	421 (31.3)	1,054 (41.6)	
Number of sexual partners				
0–1	269 (6.9)	78 (5.8)	191 (7.5)	**< 0.001**
2–5	2,400 (61.9)	929 (69.0)	1,471 (58.1)	
6+	505 (13.0)	192 (14.3)	313 (12.4)	
Unknown	704 (18.2)	147 (10.9)	557 (22.0)	
Parity				
0	68 (1.8)	12 (0.9)	56 (2.2)	**< 0.001**
1	632 (16.3)	174 (12.9)	458 (18.1)	
2	1008 (26.0)	335 (24.9)	673 (26.6)	
3	922 (23.8)	325 (24.2)	597 (23.6)	
4+	1,090 (28.1)	473 (35.1)	617 (24.4)	
Missing data	158 (4.1)	17 (2.0)	131 (5.2)	
Marital Status				
Never married	1,009 (26.0)	356 (26.5)	653 (25.8)	**< 0.001**
Married	1,941 (50.1)	682 (50.7)	1,259 (49.7)	
Previously married	919 (23.7)	303 (22.5)	616 (24.3)	
Missing data	9 (0.2)	5 (0.4)	4 (0.2)	
Place of Residence				
Rural	317 (8.2)	150 (11.1)	167 (6.6)	**< 0.001**
Urban	3,556 (91.7)	1,195 (88.8)	2,361 (93.3)	
Missing data	5 (0.1)	1 (0.1)	4 (0.2)	
HIV-status				
Negative	2,535 (65.4)	670 (47.8)	1,865 (73.7)	**< 0.001**
Positive	1,345 (34.6)	676 (50.2)	667 (26.3)	
Education Level				
None	278 (7.2)	128 (9.5)	150 (5.9)	**0.016**
Primary	903 (23.3)	358 (26.6)	545 (21.5)	
Secondary and above	2,688 (69.3)	854 (63.5)	1,834 (72.4)	
Missing data	9 (0.2)	6 (0.5)	3 (0.1)	

Controls are cancers unrelated to infection, Ever married includes widowed and divorced, IQR = Interquartile range, [#]*p* value for a t-test, [$]*p* value for the chi-squared test

South Africa, women have poorer access to health care services, are less likely to present themselves to cervical cancer screening services and have less access to cancer diagnostic facilities, which are mainly found in tertiary hospitals in urban areas [24].

The high seroprevalence of HPV16 E6 (35.9%) antibodies in cervical cancer cases found in our study are similar to the seroprevalence of HPV16 E6 (32%) in cervical cancer cases reported from the United Kingdom (35%) and India and Algeria (32%) [5, 10]. We found a relatively low

Table 2 Seroprevalence of antibodies to HPV-16 and- 18 L1, E6 and E7 proteins in infection unrelated cancer controls in HIV-negative women

Characteristics	Total	Serology markers							
				HPV-16			HPV-18		
	N = 1865	HPV-16 L1 (%)	HPV-18 L1 (%)	E6 (%)	E7 (%)	E6 & E7 (%)	E6 (%)	E7 (%)	E6 & E7 (%)
Overall seroprevalence		14.3	16.3	2.9	4.0	0.5	2.4	2.5	0.2
Demographic									
Age									
25–34	206	25 (12.1)	34 (16.5)	2 (1.0)	5(2.4)	1 (0.5)	6 (2.9)	3 (1.5)	0 (0.0)
35–44	638	86 (13.5)	93 (14.6)	19 (3.0)	21 (3.3)	4 (0.7)	17 (2.7)	12 (1.9)	1 (0.2)
45–54	1021	156 (15.3)	177 (17.3)	33 (3.2)	49 (4.8)	3 (0.3)	21 (2.1)	32 (3.1)	3 (0.3)
Chi-square trend (p value)		0.166	0.352	0.133	0.053	0.472	0.345	0.067	0.358
Place of residence									
Rural	139	18 (13.0)	20 (14.4)	9 (6.5)	12 (8.6)	2 (1.6)	3 (2.2)	11(7.9)	1 (0.8)
Urban	1772	248 (14.4)	284 (16.5)	44 (2.6)	63 (3.7)	6 (0.4)	41 (2.4)	35 (2.0)	3 (0.2)
Chi-square heterogeneity age-adjusted (p value)		0.406	0.512	0.202	**0.005**	**0.042**	0.886	**< 0.001**	0.184
Period of interview									
1995–1999	115	22 (19.1)	26 (22.6)	5 (4.4)	7 (6.1)	1 (1.0)	4 (3.5)	4 (3.5)	0 (0.0)
2000–2004	373	61 (16.4)	65 (17.4)	15 (4.0)	17 (4.6)	4 (1.2)	5 (1.3)	7 (1.9)	2 (0.6)
2005–2009	663	86 (13.0)	95 (14.3)	19 (2.9)	27 (4.1)	1 (0.2)	15 (2.3)	14 (2.1)	1 (0.2)
2010–2016	714	98 (13.7)	118 (16.5)	15 (2.1)	24 (3.4)	2 (0.3)	20 (2.8)	22 (3.1)	1 (0.16)
Chi-square heterogeneity age-adjusted (p value)		0.1757	0.123	0.202	0.455	0.142	0.368	0.538	0.454
Marital Status									
Never Married	422	56 (13.3)	67 (15.9)	11 (2.6)	12 (2.4)	2 (0.5)	5 (1.2)	11 (2.6)	0 (0.0)
Married	1002	134 (13.4)	161 (16.1)	30 (3.0)	47 (4.7)	5 (0.5)	34 (3.4)	23 (2.3)	4 (0.4)
Ever married	437	75 (17.2)	75 (17.2)	13 (3.0)	16 (3.7)	1 (0.2)	5 (1.1)	13 (3.0)	0 (0.0)
Chi-square heterogeneity age-adjusted (p value)		0.227	0.926	0.976	0.278	0.814	**0.007**	0.798	0.180
Education Level									
None	123	22 (17.9)	21(17.1)	6 (4.9)	2 (1.6)	0 (0.0)	4 (3.3)	5 (4.1)	0 (0.0)
Primary	411	54 (13.1)	61 (14.8)	9 (2.2)	20 (4.9)	1 (0.3)	10 (2.4)	8 (2.0)	1 (0.3)
Secondary and above	1329	191 (14.4)	222 (16.7)	38 (2.9)	52 (3.9)	7 (0.6)	30 (2.3)	34 (2.6)	3 (0.2)
Chi-square adjusted for age trend (p value)		0.406	0.543	0.298	0.276	0.630	0.654	0.400	0.838
Sexual/ reproductive history									
Parity									
0	34	4 (11.8)	5 (14.7)	2 (5.9)	1 (2.9)	1 (3.0)	0 (0.0)	1 (2.9)	0 (0.0)
1	316	30 (9.5)	44 (13.9)	10 (3.2)	15 (4.8)	4 (1.3)	11 (3.5)	9 (2.9)	1 (0.3)
2	485	68 (14.0)	77 (15.9)	18 (3.7)	15 (3.1)	1 (0.2)	13 (2.7)	11(2.3)	0 (0.0)
3	449	81 (18.0)	70 (15.6)	14 (3.1)	18 (4.0)	2 (0.5)	6 (1.3)	11 (2.6)	0 (0.0)
4+	486	67 (13.8)	88 (18.1)	9 (1.9)	23 (4.7)	0 (0.0)	12 (2.5)	14 (2.9)	3 (0.6)
Chi-square adjusted for age trend (p value)		0.143	0.181	**0.031**	0.931	**0.006**	0.610	0.738	0.356
Number of sexual partners									
0–1	170	22 (12.9)	25 (14.7)	6 (3.5)	6 (3.5)	0 (0.0)	1 (0.6)	2 (1.2)	0 (0.0)
2–5	1116	161 (14.4)	182 (16.3)	36 (3.2)	53 (4.8)	7 (0.7)	28 (2.5)	31(2.8)	3 (0.3)
6+	204	32 (15.7)	32 (15.7)	5 (2.6)	6 (2.9)	1 (0.5)	9 (4.4)	5 (2.5)	0 (0.0)
Chi-square adjusted for age trend (p value)		0.908	0.522	0.133	0.132	0.339	0.796	0.850	0.862
Unknown	375	52 (13.9)	65(17.3)	7 (1.9)	10 (2.7)	0 (0.0)	6 (1.6)	9 (2.4)	1 (0.3)

Table 3 Seroprevalence of antibodies to HPV-16 and- 18 L1, E6 and E7 proteins in infection unrelated cancer controls in HIV-positive women

Characteristics	Total	Serology markers							
				HPV-16			HPV-18		
	N = 667	HPV-16 L1 (%)	HPV-18 L1 (%)	E6 (%)	E7 (%)	E6 & E7 (%)	E6 (%)	E7 (%)	E6 & E7 (%)
Overall seroprevalence		15.9	15.7	2.4	3.3	0.5	1.5	3.2	0.2
Demographic									
Age									
25–34	136	24 (17.7)	15 (11.0)	5 (3.7)	5 (3.7)	1 (0.8)	1 (0.7)	1 (0.7)	0 (0.0)
35–44	300	42 (14.0)	50 (16.7)	7 (2.3)	12 (4.0)	1 (0.4)	5 (1.7)	10 (3.3)	0 (0.0)
45–54	231	40 (17.3)	40 (17.3)	4 (1.7)	5 (2.2)	1 (0.5)	4 (1.7)	10 (4.3)	1 (0.5)
Chi-square trend (p value)		0.896	0.137	0.254	0.191	0.719	0.490	0.067	0.236
Place of residence									
Rural	28	2 (7.1)	4 (14.3)	0 (0.0)	1 (3.6)	0 (0.0)	1 (3.6)	1 (3.6)	0 (0.0)
Urban	639	104 (16.3)	101 (15.8)	16 (2.5)	21 (3.3)	3 (0.5)	9 (1.4)	20 (3.1)	1 (0.2)
Chi-square heterogeneity age-adjusted (p value)		0.218	0.797	0.399	0.982	0.724	0.376	0.912	0.856
Period of interview									
1995–1999	7	1 (14.3)	1 (14.3)	1(14.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
2000–2004	90	9 (10.0)	11 (12.2)	4 (4.4)	4 (4.4)	1 (1.2)	3 (3.3)	1 (1.1)	1 (1.1)
2005–2009	230	32 (13.9)	36 (15.7	5 (2.2)	7 (3.0)	0 (0.0)	4 (1.7)	7 (3.0)	0 (0.0)
2010–2016	340	64 (18.8)	57 (16.8)	6 (1.8)	11 (3.2)	2 (0.6)	3 (0.8)	13 (3.8)	0 (0.0)
Chi-square heterogeneity age-adjusted (p value)		0.138	0.872	0.109	0.898	0.572	0.287	0.701	**0.036**
Marital Status									
Never Married	231	38 (16.5)	36 (15.6)	4 (1.7)	4 (1.7)	0 (0.0)	1 (0.4)	5 (2.2)	0 (0.0)
Married	257	32 (12.5)	35 (13.6)	8 (3.1)	12 (4.7)	1 (0.4)	4 (1.6)	8 (3.1)	0 (0.0)
Ever married	179	36 (20.1)	34 (19.0)	4 (2.2)	6 (3.4)	2 (1.2)	5 (2.8)	8 (4.5)	1 (0.6)
Chi-square heterogeneity age-adjusted (p value)		0.101	0.448	0.573	0.171	0.178	0.188	0.673	0.488
Education Level									
None	24	2 (7.4)	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Primary	134	21 (15.7)	23 (17.2)	2 (1.5)	3 (2.2)	0 (0.0)	4 (3.0)	4 (3.0)	0 (0.0)
Secondary and above	505	83 (16.4)	81 (16.0)	13 (2.6)	19(3.8)	3 (0.6)	6 (1.2)	17 (3.4)	1 (0.2)
Chi-square adjusted for age trend (p value)		0.380	0.169	0.731	0.543	0.611	0.339	0.416	0.701
Sexual/ reproductive history									
Parity									
0	22	9 (40.9)	5 (22.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	142	20 (14.1)	15 (10.6)	3 (2.1)	7 (4.9)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
2	188	38 (20.2)	30 (16.0)	6 (3.2)	8 (4.3)	2 (1.1)	3 (1.6)	8 (4.3)	1 (0.6)
3	148	17 (11.5)	27 (18.2)	3 (2.0)	5 (3.4)	1 (0.7)	4 (2.7)	7 (4.7)	0 (0.0)
4+	131	17 (13.0)	21 (16.0)	3 (2.3)	2 (1.5)	0 (0.0)	2 (1.5)	4 (3.1)	0 (0.0)
Chi-square adjusted for age trend (p value)		0.022	0.688	0.614	0.340	0.932	0.294	0.347	0.373
Number of sexual partners									
0–1	21	3 (14.3)	4 (19.1)	1 (4.8)	2 (9.5)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
2–5	355	55 (15.5)	52 (14.7)	5 (1.4)	9 (2.5)	0 (0.0)	8 (2.3)	8 (2.3)	1 (0.3)
6+	109	20 (18.4)	19 (17.4)	5 (4.6)	3 (2.8)	1 (1.0)	1 (0.9)	3 (2.8)	0 (0.0)
Chi-square adjusted for age trend (p value)		0.869	0.875	0.260	0.518	0.041	0.143	0.291	0.602
Unknown	182	28 (15.4)	30 (16.5)	5 (2.8)	8 (4.4)	2 (1.2)	1 (0.6)	9 (5.0)	0 (0.0)

Table 4 Association between HPV-16 and -18 L1 and (E6 and E7) seropositivity and cervical cancer

Serology markers	Cases (N = 1,346) n (%) Seropositive	Controls (N = 2,532) n (%) Seropositive	Adjusted OR (95% CI)
HPV16 L1	332 (24.4)	373 (14.7)	1.74 (1.45–2.07)
HPV18 L1	292 (21.7)	409(16.2)	1.31 (1.10–1.56)
HPV type 16			
HPV16 E6	467 (35.9)	70 (2.8)	21.96 (16.61–29.02)
HPV16 E7	309 (24.7)	97 (3.8)	7.93 (6.16–10.22)
HPV16 E6 & E7	209 (23.3)	11 (0.5)	69.20 (37.07–129.18)
HPV type 18			
HPV18 E6	120 (8.9)	54 (2.1)	4.67 (3.30–6.59)
HPV18 E7	235 (18.6)	68 (2.7)	8.94 (6.64–12.03)
HPV18 E6 &E7	64 (5.9)	5 (0.2)	38.61 (15.13–98.53)
HPV types 16 or 18			
HPV16/18 E6/E7	746 (56.8)	255 (10.1)	13.63 (11.32–16.41)

OR adjusted for age, HIV antibodies, education, number of sexual partners, place of residence, marital status and period of interview

seroprevalence of HPV16 E6 & E7 and HPV18 E6 &E7 antibodies among controls in keeping with a low prevalence of HPV oncoprotein antibodies among women without cervical cancer [25]. Our study demonstrates that the high seroprevalence of HPV16 E6 and E7 antibodies in cervical cancer development is in accordance with other studies from Russia and Italy [8, 26].

Antibodies to the E6 and E7 oncoproteins are late markers of invasive cervical cancer [27]. In our study, seropositivity to each of the HPV-16 and -18 (E6 and E7) antibodies was associated with high risks of cervical cancer. Notably, seropositivity to HPV16 (E6 & E7) and HPV18 (E6 & E7) exhibited the highest ORs for cervical cancer (Fig. 3). Similar findings were reported in a case–control study of patients recruited from India and Algeria [10]. Our findings support those from other cross-sectional, case–control and prospective studies, albeit each using different assays [27–30].

Individuals with suppressed immune systems are susceptible to chronic infection, including HPV [31]. Thus, HIV infection increases the risk of HPV persistence [32]. Existing evidence of the association between HPV (E6 and E7) and L1 proteins antibodies and HIV has come from studies on men who have sex with men [10, 33, 34]. In contrast, most of the existing studies on the association between antibodies to HPV-16 and -18 L1, E6 and E7 proteins and cervical cancer risk have been conducted in low HIV prevalence settings [6, 8, 13, 27]. In our study, access to HIV data made it possible to stratify our analysis by HIV status. Our finding showed that antibody seropositivity to each of HPV-16 and -18 (E6 and E7) oncoproteins was strongly associated with the risk of cervical cancer among HIV-positive women. Our data support the hypothesis that HIV plays an important role in the persistence of HPV infection.

Unexpected findings in our study were that HPV-16 and -18 (E6 and E7) oncoproteins seropositivity and cervical cancer risk were higher in HIV-negative cancer patients compared to HIV-positive cancer patients. Similar findings have been observed in previous studies of HPV antibodies among HIV-positive and HIV-negative patients [35–38]. In our study, the possible explanation could be that cervical cancer risk in HIV positive women may be related to more than the two main types HPV16 and HPV18 that we tested for so their relative contribution appears attenuated (Additional file 1: Table S3). Other studies on HPV genotype distribution by HIV-status that were conducted in South Africa reported that HPV types 35, 58 and 33 were more commonly identified [39, 40] in addition to types 16 and 18. However, if this is indeed true, then the current HPV vaccine (16/18) may be less effective for HIV positive women. Another possible explanation is that in HIV-positive patients, antibodies could be an indication of reactivation of a latent HPV while in HIV-negative patients antibodies could be reinfection with a new type [35]. Another unexpected finding in our study was that the number of sexual partners was not associated with HPV L1 and (E6 and E7) antibodies. The plausible explanation could be that peak acquisition of HPV occurs at an age earlier than the age of participants in our study [41].

In the new guidelines for screening cervical cancer, the World Health Organization (WHO) has included HPV antibodies and oncoproteins as a future potential screening test [42]. We, therefore, assessed the clinical performance of HPV E6 /E7 antibodies as possible

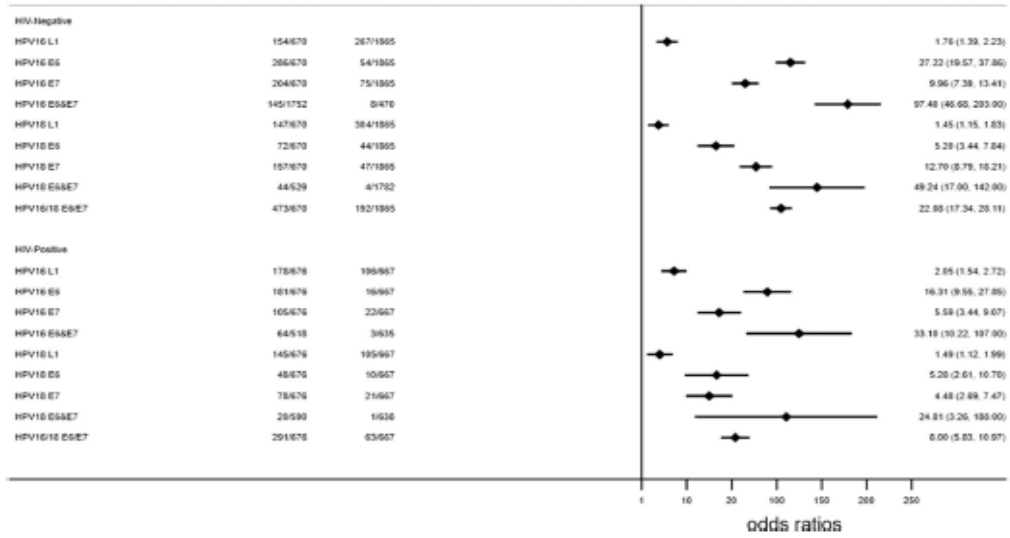

Fig. 3 Relationship between HPV-16 and -18 (L1, E6 and E7) seropositivity and cervical cancer risk by HIV-status. Odds ratios adjusted for age, education level, number of sexual partners, marital status place of residence and period of interview

screening tests. We found that the sensitivity of the HPV antibodies for detection of cervical cancer was low but the specificity was high among HIV-positive women. In contrast, sensitivity and specificity for the detection of cervical cancer were high among HIV-negative women. Predictive values would thus vary considerably depending on the background prior likelihood of disease. Our finding is in line with the previous findings on the sensitivity and specificity of HPV16/18 E6/E7 antibodies as a screening test [28, 43].

There are strengths to our study. The seroprevalence of HPV antibodies was conducted on a large sample size, and we used a high throughput multiplex serology so all tests were done under the same laboratory conditions. The laboratory tests were conducted 'blind' without prior knowledge of the case/control status of the participants. Nonetheless, our study has some limitations. Serology data on exposure and cervical cancer diagnoses (of all stages) were collected at the same time. We did not have tumor HPV Deoxyribonucleic

Table 5 Clinical performance of HPV16 and 18 antibody positivity as diagnostic markers for cervical cancer by HIV-status

Serology markers	HIV-positive			HIV-negative		
	Sensitivity (95% CI)	Specificity (95% CI)	AUC	Sensitivity (95% CI)	Specificity (95% CI)	AUC
HPV16 E6	26.8 (23.5–30.3)	97.6 (96.1–98.6)	62	42.7 (38.9–46.5)	95.6 (94.5–96.4)	69
HPV16 E7	15.5 (12.9–18.5)	96.7 (95.0–97.9)	56	30.4 (27.0–34.1)	96.0 (95.0–96.8)	63
HPV16 E6 & E7	12.4 (9.6–15.5)	99.5 (98.6–99.9)	56	30.9 (26.7–35.2)	99.5(99.1–99.8)	65
HPV18 E6	7.1 (5.3–9.3)	98.5 (97.3–99.3)	53	10.7 (8.5–13.3)	97.6 (96.8–98.3)	54
HPV18 E7	11.5 (9.2–14.2)	97.9 (95.2–98.0)	54	23.4 (20.3–26.8)	97.1 (96.4–97.7)	60
HPV18 E6 & E7	3.4 (2.1–5.2)	99.8 (99.1–100)	52	8.3 (6.1–11.0)	99.9 (99.5–99.9)	54
HPV16/18 E6/E7	43.0 (39.3–46.9)	90.6 (88.1–92.7)	67	70.6 (67.0–74.0)	89.7(88.2–91.0)	80

Acid (DNA) data to determine the causative type(s). Since only 50–70% of women with detectable HPV DNA in the cervix seroconvert [44], our study underestimates the true prevalence of HPV infection. The JCS did not recruit women with cervical pre-cancerous lesions. Study results are based on black women aged 25- 54 years who were recruited from the catchment area of the largest public tertiary hospital in Johannesburg. Therefore, our findings might not be generalizable to other South African regions and women aged 55 years and above.

Conclusions

Our data contribute to the evidence on the importance of HPV-16 and -18 E6 and E7 oncoproteins antibodies in discriminating cervical cancer from controls in a black South African population. HPV L1 antibodies show to be exposure markers of past HPV infection. HPV E6 and E7 show to be important markers of invasive cervical cancer. Furthermore, antibodies to HPV-16 and-18 E6 and E7 seropositivity are strongly associated with cervical cancer in both HIV-positive and HIV-negative patients.

Abbreviations

AOR: Adjusted odds ratio; AUC: Area under the curve; CI: Confidence interval; DFKZ: Deutsches Krebsforchungszentrum; DNA: Deoxyribonucleic acid; ELISA: Enzyme-linked immunosorbent Assay; EDTA: Ethylenediaminetetraacetic acid; GST: Glutathione S-transferase; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; IQR: Interquartile range; JCS: Johannesburg Cancer Study; SST: Serum separation tube; MFI: Median fluorescence intensity; ROC: Receiver operating characteristic; USA: United States of America; VIP: Visual inflection point; WHO: World Health Organization.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13027-022-00418-2.

Additional file 1: Table S1: Comparison of HPV16 and 18 (E6 and E7, L1) antibodies in cervical cancer Cases and Controls among young and older women. **Table S2:** Seroprevalence of HPV16 and 18 L1. **Table S3:** Seroprevalence of antibodies against HPV 16 and 18 (L1, E6 and E7) by HIV-Status. **Table S4:** Clinical performance of HPV16 and 18 antibodies as a diagnostic marker for cervical cancer. **Fig. S1:** Age-adjusted seroprevalence of HPV related antibody markers in cervical cancer cases and other infection unrelated cancer controls and p-value for heterogeneity among the infection unrelated cancer controls (i.e. breast, colon, oesophagus, endometrium lung, pancreas, other minor types). The seropositivity of the age-adjusted HPV L1 and (E6 and E7) antibodies in each of the infection unrelated cancer patients (controls) was similar, (p-heterogeneity > 0.05). **Fig. S2:** Correlation between HPV16 and 18 proteins L1, E6 and E7 antibodies and HIV antibodies, R-values > 0.8 shows high correlation. (* Significance at 0.05). The colour indicates the intensity of correlation. Green indicate positive values, red indicate negative values, orange shows R-values that are greater than 0.05 and dark orange indicate R-values < 0.05 but not statistically significant.

Acknowledgements

We thank all those who provided feedback to improve the manuscript.

Authors' contributions

MGS conceptualised the study. MGS performed data analysis and drafted the manuscript. FS, TW, CBdeV and ES edited the manuscript. NB and TW conducted the laboratory analyses. WCC was responsible for data acquisition, data curation quality controls, and management. DB, TW, CGM, FS, ES, MMo, MMu, ABK and RN are the members of the ERICA_SA collaborative study. All authors read and provided feedback to improve the final version of the manuscript.

Funding

This study was supported by the South African Medical Research Council (with funds received from the South African National Department of Health) and the UK Medical Research Council (with funds from the UK Government's Newton Fund) (MRC-RFA-SHIP 01- 2015). The funders were not involved in the conceptualization, review or approval of the manuscript. This project forms part of an international research program aimed at identifying evolving risk factors for cancer in African populations (ERICA-SA). (https://www.samrc.ac.za/intramural-research-units/evolving-risk-factors-cancers-african-populations-erica-sa).

Availability of data and materials

Data cannot be shared publicly because of ethics policy at University of Witwatersrand, whereby any new analyses require Human Research Ethics Committee approval. Data are available from the SA-NCR /National Health Laboratory Services. (contact: adrianp@nicd.ac.za) for researchers who meet the relevant ethics criteria for access to these data.

Declarations

Ethics approval and consent to participate

The JCS and the current study were approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number for the current study. M200252). In the JCS, participants gave written informed or wittnessd consent to once-off interview and optional blood draw and to have their information and blood sample anonymized. Any future investigations require approval of the University of the Witwatersrand Human Research Ethics Committee HREC.

Consent for publication

Not applicable.

Competing interests

Freddy Sitas is a member of the Editorial Board of Infectious Agents and Cancer journal but had no involvement in the review process of this manuscript.

Author details

[1]National Cancer Registry, National Health Laboratory Service, Sandringham, Johannesburg, South Africa. [2]Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [3]Burden of Disease Research Unit, South African Medical Research Council, Cape Town, South Africa. [4]Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [5]Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [6]Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College London, London SE1 9RT, UK. [7]MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda. [8]University of York, York, UK. [9]South African DSI-NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), Stellenbosch University, Stellenbosch, South Africa. [10]Division of Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [11]Centre for Primary Health Care and Equity, School of Population Health, University of New South Wales, Sydney, Australia. [12]Menzies Centre of Health Policy, School of Public Health, University of Sydney, Sydney, Australia.

Received: 3 December 2021 Accepted: 11 February 2022
Published online: 29 March 2022

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Publisher's Note

PLOS ONE

RESEARCH ARTICLE

Ranking lifestyle risk factors for cervical cancer among Black women: A case-control study from Johannesburg, South Africa

Mwiza Gideon Singini[1,2], Freddy Sitas[3,4,5], Debbie Bradshaw[3], Wenlong Carl Chen[1,6], Melitah Mothale[1,2], Abram Bunya Kamiza[6], Chantal Babb de Villiers[7], Cathryn M. Lewis[8,9], Christopher G. Mathew[6,9], Tim Waterboer[10], Robert Newton[11,12], Mazvita Muchengeti[1,2,13], Elvira Singh[1,2]*

1 National Cancer Registry, National Health Laboratory Service, Johannesburg, South Africa, **2** Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa, **3** Burden of Disease Research Unit, South African Medical Research Council, Cape Town, South Africa, **4** Centre for Primary Health Care and Equity, School of Public Health and Community Medicine, University of New South Wales Sydney, Australia, **5** Menzies Centre of Health Policy, School of Public Health, University of Sydney, Australia, **6** Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, **7** Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, **8** Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, **9** Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College, London, United Kingdom, **10** Division of Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, **11** MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda, **12** University of York, York, United Kingdom, **13** South African DSI-NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), Stellenbosch University, Stellenbosch, South Africa

* elviras@nicd.ac.za

OPEN ACCESS

Citation: Singini MG, Sitas F, Bradshaw D, Chen WC, Mothale M, Kamiza AB, et al. (2021) Ranking lifestyle risk factors for cervical cancer among Black women: A case-control study from Johannesburg, South Africa. PLoS ONE 16(12): e0260319. https://doi.org/10.1371/journal.pone.0260319

Editor: Nülüfer Erbil, Ordu University, TURKEY

Received: June 9, 2021

Accepted: November 7, 2021

Published: December 8, 2021

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0260319

Data Availability Statement: Data cannot be shared publicly because of ethics policy at University of Witwatersrand, whereby any new analyses require Human Research Ethics

Abstract

Background

Aside from human papillomavirus (HPV), the role of other risk factors in cervical cancer such as age, education, parity, sexual partners, smoking and human immunodeficiency virus (HIV) have been described but never ranked in order of priority. We evaluated the contribution of several known lifestyle co-risk factors for cervical cancer among black South African women.

Methods

We used participant data from the Johannesburg Cancer Study, a case-control study of women recruited mainly at Charlotte Maxeke Johannesburg Academic Hospital between 1995 and 2016. A total of 3,450 women in the study had invasive cervical cancers, 95% of which were squamous cell carcinoma. Controls were 5,709 women with cancers unrelated to exposures of interest. Unconditional logistic regression models were used to calculate adjusted odds ratios (OR_{adj}) and 95% confidence intervals (CI). We ranked these risk factors by their population attributable fractions (PAF), which take the local prevalence of exposure among the cases and risk into account.

Committee approval. Data are available from the SA-NCR /National Health Laboratory Services. Contact adrianp@nicd.ac.za for researchers who meet the relevant ethics criteria for access to these data.

Funding: This study was supported by the South African Medical Research Council (with funds received from the South African National Department of Health) and the UK Medical Research Council (with funds from the UK Government's Newton Fund) (MRC-RFA-SHIP 01-2015). The funders were not involved in the conceptualisation, review or approval of the manuscript. This project forms part of an international research program aimed at identifying evolving risk factors for cancer in African populations (ERICA-SA). (https://www.samrc.ac.za/intramural-research-units/evolving-risk-factors-cancers-african-populations-erica-sa).The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Results

Cervical cancer in decreasing order of priority was associated with (1) being HIV positive (OR_{adj} = 2.83, 95% CI = 2.53–3.14, PAF = 17.6%), (2) lower educational attainment (OR_{adj} = 1.60, 95% CI = 1.44–1.77, PAF = 16.2%), (3) higher parity (3+ children vs 2–1 children (OR_{adj} = 1.25, 95% CI = 1.07–1.46, PAF = 12.6%), (4) hormonal contraceptive use (OR_{adj} = 1.48, 95% CI = 1.24–1.77, PAF = 8.9%), (5) heavy alcohol consumption (OR_{adj} = 1.44, 95% CI = 1.15–1.81, PAF = 5.6%), (6) current smoking (OR_{adj} = 1.64, 95% CI = 1.41–1.91, PAF = 5.1%), and (7) rural residence (OR_{adj} = 1.60, 95% CI = 1.44–1.77, PAF = 4.4%).

Conclusion

This rank order of risks could be used to target educational messaging and appropriate interventions for cervical cancer prevention in South African women.

Introduction

Cervical cancer is the fourth most frequently occurring cancer type and the fourth leading cause of death from cancer among women globally [1]. In sub-Saharan Africa (SSA), more than 101,423 new cases and 76,444 deaths of cervical cancer occur each year [1]. In South Africa, the age-standardised incidence rate of cervical cancer as reported by Globocan is 44.4 per 100,000 women per year [1]. According to the 2017 South African National Cancer Registry report, there were 5,630 new histologically confirmed cases of cervical cancer amongst black women with a lifetime risk (0–74 years) of 1 in 33 women [2].

The main risk factor for cervical cancer and its premalignant lesions is persistent infection with high-risk Human papillomaviruses (hr-HPV) [3]. A previous study from the Johannesburg Cancer Study (JCS), showed a seroprevalence of 78% for anti HPV-16 antibodies in cervical cancer cases [4]. However, at least 90% of women with hr-HPV infection, do not develop cervical cancer [3]. This suggests that other environmental cofactors acting jointly with hr-HPV elevate the risk of cervical carcinogenesis [5].

Epidemiological studies have indicated human immunodeficiency virus (HIV), parity, smoking, alcohol consumption, contraceptive use, age, education and number of sexual partners to be cofactors of cervical cancer pathogenesis [5–12], but these cofactors vary in prevalence (and possibly the risk) in different settings. Understanding the prevalence levels of different cofactors and their risk for cervical cancer in a local setting may help in identifying risk profiles to target in cervical cancer prevention.

In South Africa, studies on some lifestyle-related cofactors for cervical cancer were published about 10 years ago from the JCS [13–15]. These studies focused separately on individual cofactors such as HIV [15], smoking [14] or contraceptive use [13]. Among black South African women little is known about the relative importance of these cofactors for cervical cancer.

In South Africa, the JCS was established in 1995 to redress at that time the historical paucity of epidemiological cancer research among black South Africans [16]. The JCS aimed to examine whether key known and emerging risk factors for cancer in women of mainly European ancestry applied to patients of African ancestry in Johannesburg, South Africa. These aims evolved into measuring the importance of known and emerging risk factors for cancer in a local setting.

We analysed JCS lifestyle data to evaluate the contribution of different cofactors in the pathogenesis of cervical cancer among black South African women. In the current study, we used a

subset of the JCS female samples and focused on ages 25 to 64 years to align with current cervical screening guidelines but also allowing for a more comparable assessment of the contribution of different cofactors in rank order to the risk of cervical cancer.

Methods

Setting and participants

The details of the JCS have been described elsewhere [16], but briefly, the JCS recruited over 26,000 black South African patients (both sexes) who were newly diagnosed with an incident cancer between 1995 and 2016. Recruitment took place mainly at Charlotte Maxeke-Johannesburg Academic Hospital medical oncology and radiotherapy clinics and associated peripheral clinics. Trained interviewers collected self-reported data on demographics and key lifestyle risk factors from consenting participants using a structured questionnaire, and took peripheral blood samples for HIV and other analyses. The questionnaire included questions on the following: socio-demographic factors such as; place of birth and residence, marital status, education, the home language of parents. Enviromental exposures such as method of cooking and heating. Lifestyle factors such as; smoking by type of tobacco and amounts smoked, snuff (sniffed tobacco) use, alcohol consumption by type, parity, use of oral and injectable contraceptives, number of sexual partners. On occupations, self-reported use of Anti-Retroviral Therapy (ART) (since 2005), PAP smear (2001) and self-reported history of diabetes. The JCS and the current study were approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number for the current study. M200252). In the JCS, participants gave written informed or wittnessd consent to once-off interview and optional blood draw and to have their information and blood sample anonymized. Any future investigations require approval of the University of the Witwatersrand Human Research Ethics Committee HREC [16].

Selection of cases and controls

The JCS is amenable to a case-control design and analysis. Cases were women who were newly diagnosed with invasive cervical cancer (histologically confirmed squamous cell carcinoma and adenocarcinoma) and controls were designated as women with newly diagnosed cancers that have no known relationship with the exposures of interest [16].

A total of, 26,263 participants were enrolled in the JCS between 1995 to 2016. We excluded 8,677 (33.0%) males, 3,105 (17.6%) women older than 65 years or younger than 25 years, 663 (4.6%) non-cancer participants, 945(6.9%) non-South Africans, 1 with missing data, 640 (5.0%) with missing HIV-status and 691 (5.7%) with primary site unknown malignancy. From those with cancer of the cervix, we excluded International Classification of Diseases for Oncology (ICD-O) codes such as (ICD-O morphology: 8010–8050 epithelial neoplasm (n = 98, 2.6%), (ICD-O morphology: 8000 and 8001) not otherwise specified (n = 9, 0.2%), (ICD-O morphology: 8560, 8570 and 8574) complex epithelial neoplasms (n = 108, 2.8%) and other minor histological types (ICD-O morphology: 8098,8170, 8200, 8272, 8310, 8441,8460, 8480–8490, 8500,8650 and 8931–9100) (n = 64, 1.7%) Fig 1.

Fig 1 also outlines the criteria used to select cases and controls. The control groups were arranged into four sets. Each set comprised of women diagnosed with different cancer types that are unrelated to the exposures of interest (these being infection, smoking, alcohol, parity, number of sexual partners and use of hormonal contraceptives) [16–18] Fig 1. Cancer controls unrelated to infection constituted the highest number of controls S1 Table. The demographic analyses excluded 2,009 (26.0%) women with cancers related to infections. Analyses relating to smoking excluded 2,218 (28.7%) women with cancers related to smoking and infections.

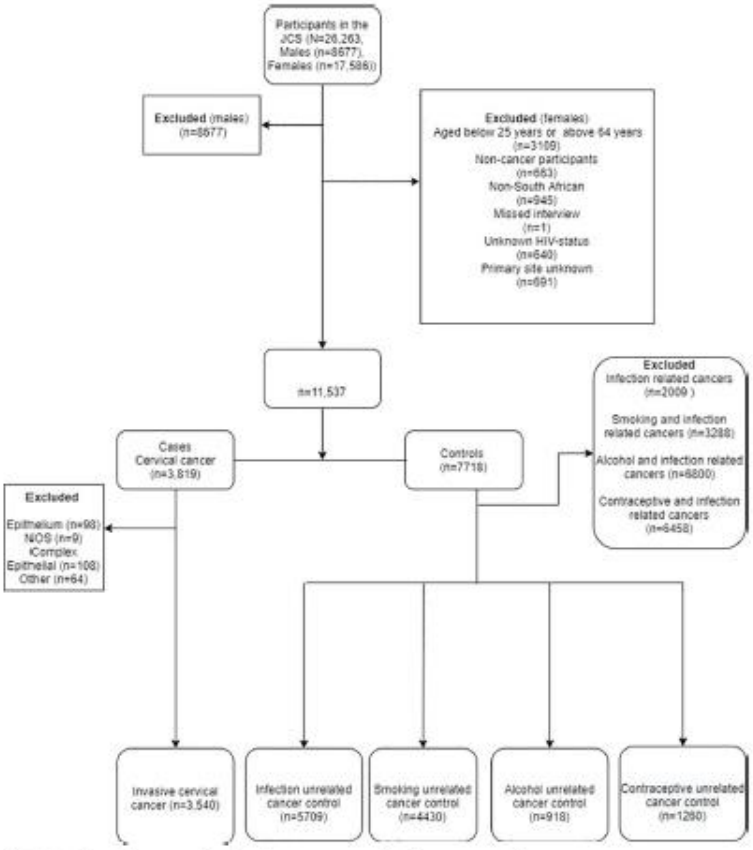

Fig 1. Data flow diagram on the selection exposure unrelated cancer controls.

https://doi.org/10.1371/journal.pone.0260319.g001

Analyses relating to alcohol excluded 6,800 (60.4%) women with cancers related to alcohol and infections. Analyses relating to hormonal contraceptives excluded 6,458 (57.4%) women with cancers related to hormonal contraceptives and infections. Of the remaining 9,249 women, 3,540 (38.3%) had invasive cervical cancer (defined as cases) and 5,709 (61.7%) had other cancers (defined as controls) that were not related to the exposure of interest Fig 1.

Definition of exposures

For other demographic factors, women were grouped according to the period of the interview (1995–1999, 2000–2004, 2005–2009, 2010–2016), education level (none, primary, secondary and tertiary and unknown), marital status (never married, married, ever married and unknown) and place of residence (rural, urban and unknown).

Women's HIV status (positive and negative) was based on Abbott Axysm HIV1/2 gO Microparticle Enzyme-linked Immunoassay (ELISA) (1995 to 2005) and the Vironostika (HIV UniForm II plus O) micro ELISA (2006 to 2016) test results [15], while the other cofactors

were self-reported. Use of hormonal contraceptive was categorized into (never (injectable and oral), ever oral/ not injectable, ever injectable/ not oral, ever oral, injectable and ever injectable and/ or oral) and unknown [13]. Parity was categorized into 1–2, > = 3 children and unknown. We excluded 168 (1.9%) women who had never given birth (on assumption that some may have had pre-existing unreported gynaecological conditions or hysterectomies). The number of sexual partners for the participants were grouped into 0–1, 2–5, > = 6 and unknown.

Women's smoking status was classified into never (non-smokers), ex-smoker, current smoker and unknown. The current smokers included those women who reported to have quit smoking within 5 years of interview (diagnosis) to reduce the likelihood of reverse causation [14]. We calculated total alcohol consumption based on different types of alcoholic beverages consumed: spirits (homebrew and commercial), beer (homebrew and commercial), wine, maize and sorghum, the content of ethanol from each type of alcohol beverages and the number of drinks per day. The average number of drinks per week was used to calculate the total alcohol consumption [19]. We defined one drink as to have drunk 15.7 grams of unmixed alcohol beverage, which is equivalent to one bottle of beer of 340 ml that contains 4% of ethanol. Based on the Center for Disease Control (CDC) definitions [20], we categorized women's self-reported consumption of alcoholic beverages into never, light (31.4 grams or less of alcohol per week) and heavy (47.1 grams or more of alcohol per week).

Statistical analysis

Demographic characteristics were summarized using frequencies and percentages for the categorical variables, medians and the interquartile range (IQR) for the continuous variable that was not normally distributed. We then analyzed the relationship between each of the six cofactors with cervical cancer, using the appropriate set of cancer controls for each analysis Fig 1.

We assessed for HIV status in model 1, parity in model 2, smoking in model 3, alcohol consumption in model 4; hormonal contraceptive use in model 5, and the number of sexual partners in model 6. We fitted our data using a backward stepwise multivariable unconditional logistic regression to estimate the unadjusted and adjusted odds ratios (OR_{adj}) and 95% confidence intervals (CI). In the final multivariable analysis of each model, we returned to the model variables where literature supported an association with cervical cancer. We ran 6 different models with different main exposures. We adjusted simultaneously for the potential confounders: age (grouped), the period of the interview, marital status, place of residence and education level. We performed a test for heterogeneity on categorical variables and a score test for trends in the odds ratios on ordinal categorical variables using unconditional logistic regression Table 2. We used a Pearson correlation matrix to test for multicollinearity between independent variables in each model [21]. We used the Mantel-Haesnzel Chi-square test to assess differences in the prevalence of cofactors across different cancer types in different sets of controls Table 3.

Population attributable fractions

We calculated population attributable fraction (PAF) using Miettinen's method given by $PAF = P_{ei}\left(\frac{OR_{adj}-1}{OR_{adj}}\right)$ [22]; where P_{ei} is the proportion of cases exposed to a particular cofactor and i is the exposure level. When a variable had three categories, we added the PAF for the categories. A PAF % represents the proportion of the disease attributable to a particular exposure. We then ranked the cofactors which were significant based on their PAF. All the statistical tests were done using STATA software version 16.0 (Stata Corp, college station, Tx). Statistical significance was considered at a two-sided α-level of 0.05.

Results

Controls were relatively older compared to cases (Median age 50 years (IQR: 42–57) versus 48 years (IQR: 41–55)). The highest percentage of participants were in the age group of 45–54 years: 36.4% among cervical cancer cases and 33.8% among controls. Most of the study participants (cases = 84.2% and controls = 90.4%) lived in urban areas. More than half of the participants (58.5%) achieved a secondary or greater level of education Table 1.

Cases had nearly three times the risk of being HIV positive compared to controls (OR_{adj} 2.83, 95% CI: 2.54–3.15). After controlling for the common set of confounders, a strong trend of elevated risk with higher parity was observed, relative to 1–2 children, with odds ratios of

Table 1. Demographics: Cervical cancer cases and infection unrelated cancer control participants from the JCS.

Characteristics	Total (N = 9249 (100%)) N (%)	Cases (N = 3540 (100%)) n (%)	Controls (N = 5709 (100%)) n (%)
Cervical cancer types			
SCC		3364 (95.0)	
Adenocarcinoma		176 (5.0)	
Demographics			
Age			
Median (IQR)	49 (42–56)	48 (41–55)	50 (42–57)
Age			
25–34	795 (8.6)	286 (8.1)	525 (8.9)
35–44	2410 (26.1)	1010 (28.5)	1400 (24.5)
45–54	3220 (34.8)	1288 (36.4)	1932 (33.8)
55–64	2824(30.5)	956 (27.1)	1868 (32.7)
Period of interview			
1995–1999	1619 (17.5)	766 (21.6)	853 (14.8)
2000–2004	1455 (15.7)	477 (13.5)	978 (17.1)
2005–2009	2544 (27.5)	994 (28.1)	1550 (27.2)
2010–2016	3631 (39.3)	1303 (36.8)	2328 (40.8)
Marital Status			
Never Married	2224 (24.1)	861 (24.3)	1363 (23.9)
Married	4152 (44.8)	1581 (44.7)	2571 (45.0)
Ever married	2848 (30.8)	1088 (30.7)	1760 (30.8)
Missing data	25 (0.3)	10 (0.3)	15 (0.3)
Place of Residence			
Rural	1069 (11.5)	546 (15.4)	524 (9.1)
Urban	8181 (88.1)	2982 (84.2)	5190 (90.4)
Missing data	39 (0.4)	13 (0.4)	26 (0.5)
Education Level			
None	1037 (11.2)	515 (14.6)	522 (9.1)
Primary	2772 (30.0)	1202 (34.0)	1570 (27.5)
Secondary	4930 (53.3)	1707 (48.2)	3223 (56.5)
Tertiary	479 (5.2)	97 (2.7)	379 (6.6)
Missing data	34 (0.4)	19 (0.5)	15 (0.3)

Cancer Controls are unrelated to infection, Ever married includes widowed and divorced, IQR = Interquartile range, SCC = Squamous Cell Carcinoma, JCS = Johannesburg Cancer Study

https://doi.org/10.1371/journal.pone.0260319.t001

Table 2. Multivariable analysis of lifestyle risk factors and cervical cancer participants from the JCS.

Characteristics	Total (N = 9249) N (%)	Cases (N = 3540) n (%)	Controls (N = 5709) n (%)	Unadjusted OR (95%CI)	Adjusted Odds Ratios (95% CI) using appropriately chosen controls	Case control comparison p-value
Demographic factors						
Age						
25–34	795 (8.6)	286 (8.1)	525 (8.9)	1.10 (0.93–1.29)	1.00 (0.96–1.37)	0.906
35–44	2410 (26.1)	1010 (28.5)	1400 (24.5)	1.41 (1.26–1.58)	**1.34 (1.17–1.52)**	**<0.001**
45–54	3220 (34.8)	1288 (36.4)	1932 (33.8)	1.30 (1.17–1.45)	**1.28 (1.14–1.43)**	**<0.001**
55–64	2824(30.5)	956 (27.1)	1868 (32.7)	1.00	1.00	
p-value (trend)				<0.001	0.016	
Period of interview						
1995–1999	1619 (17.5)	766 (21.6)	853 (14.8)	1.00	1.00	
2000–2004	1455 (15.7)	477 (13.5)	978 (17.1)	0.54 (0.47–0.63)	**0.53 (0.45–0.62)**	**<0.001**
2005–2009	2544 (27.5)	994 (28.1)	1550 (27.2)	0.71 (0.63–0.81)	**0.67 (0.59–0.77)**	**<0.001**
2010–2016	3631 (39.3)	1303 (36.8)	2328 (40.8)	0.62 (0.55–0.70)	**0.59 (0.51–0.68)**	**<0.001**
p-value (trend)				<0.001	<0.001	
Marital Status						
Never Married	2224 (24.1)	861 (24.3)	1363 (23.9)	1.00	1.00	
Married	4152 (44.8)	1581 (44.7)	2571 (45.0)	0.99 (0.87–1.12)	**1.14 (1.01–1.28)**	**0.023**
Ever married	2848 (30.8)	1088 (30.7)	1760 (30.8)	1.00 (0.87–1.14)	1.11 (0.98–1.27)	0.061
Missing data	25 (0.3)	10 (0.3)	15 (0.3)	-	-	
p-value (heterogeneity)				0.880	0.156	
Place of residence						
Rural	1069 (11.5)	546 (15.4)	524 (9.1)	1.85 (1.62–2.01)	**1.62 (1.41–1.86)**	**<0.001**
Urban	8181 (88.1)	2982 (84.2)	5190 (90.4)	1.00	1.00	
Missing data	39 (0.4)	13 (0.4)	26 (0.5)	-	-	
p-value (heterogeneity)				<0.001	<0.001	
Education Level						
None	1037 (11.2)	515 (14.6)	522 (9.1)	1.97 (1.72–2.25)	**2.01 (1.73–2.34)**	**<0.001**
Primary	2772 (30.0)	1202 (34.0)	1570 (27.5)	1.53 (1.39–1.68)	**1.60 (1.44–1.77)**	**<0.001**
Secondary and above	5406 (58.5)	1804 (51.0)	3223 (63.1)	1.00	1.00	
Missing data	34 (0.4)	19 (0.5)	15 (0.3)			
p-value (heterogeneity)				<0.001	<0.001	
Lifestyle factors						
HIV Status					**Model 1**	
Negative	6759 (72.8)	2217 (62.6)	4542 (79.0)	1.00	1.00	
Positive	2550 (27.2)	1323 (37.4)	1207 (21.0)	2.32 (2.10–2.54)	**2.83 (2.53–3.14)**	**<0.001**
Missing data	-	-	-		-	
p-value (heterogeneity)				<0.001	<0.001	
Parity					**Model 2**	
1–2	1595 (33.2)	1138 (32.2)	457 (35.5)	1.00	1.00	
3+	3021 (668.9)	2288 (65.0)	733 (57.0)	1.49 (1.36–1.63)	**1.25 (1.07–1.46)**	**0.005**
Missing data	190 (4.0)	94 (2.7)	96 (7.5)	0.65 (0.50–0.83)	**0.57 (0.44–0.74)**	**0.002**
p-value (trend)				<0.001	0.002	
Smoking					**Model 3**	

(*Continued*)

Table 2. (Continued)

Characteristics	Total (N = 9249) N (%)	Cases (N = 3540) n (%)	Controls (N = 5709) n (%)	Unadjusted OR (95%CI)	Adjusted Odds Ratios (95% CI) using appropriately chosen controls	Case control comparison p-value
Never	6737(83.3)	2830 (79.9)	3807 (85.9)	1.00	1.00	
Ex-Smoker	424 (5.3)	209 (5.9)	215 (4.8)	1.31 (1.07–1.59)	**1.26 (1.02–1.55)**	**0.030**
Current	901 (11.3)	498 (14.1)	403 (9.2)	1.66 (1.44–1.91)	**1.55 (1.34–1.80)**	**<0.001**
Missing data	8 (0.1)	3 (0.1)	5 (0.1)	-	-	
p-value (heterogeneity)				<0.001	<0.001	
Alcohol consumption					**Model 4**	
Never	3538 (79.3)	2781 (78.6)	757 (82.5)	1.00	1.00	
Light	243 (5.5)	194 (5.5)	49 (5.3)	1.08 (0.77–1.49)	1.20 (0.86–1.68)	0.287
Heavy	677 (15.2)	565 (16.0)	112 (12.2)	1.37 (1.10–1.71)	**1.44 (1.15–1.81)**	**0.002**
Missing data	-	-	-	-	-	
p-value (heterogeneity)				0.017	0.078	
Contraceptive use					**Model 5**	
Never (injectable and oral)	1833 (38.2)	1262 (35.7)	571 (45.3)	1.00	1.00	
Ever Oral/not injectable	578 (12.0)	408 (11.5)	170 (13.5)	1.09 (0.89–1.33)	1.09 (0.88–1.36)	0.847
Ever Injectable/ not oral	1526 (31.8)	1226 (34.5)	305 (24.2)	1.81 (1.54–2.13)	**1.34 (1.11–1.61)**	**0.002**
Ever oral and Injectable	844 (17.6)	637 (18.0)	207 (16.4)	1.39 (1.16–1.68)	1.22 (1.00–1.50)	0.366
Ever injectable and/ or oral	2948 (61.4)	2266 (64.0)	709 (54.5)	1.50 (1.32–1.71)	**1.17 (1.01–1.37)**	**0.039**
Missing data	19 (0.4)	12 (0.3)	7 (0.6)	-	-	
p-value (heterogeneity)				<0.001	0.183	
Number of sexual partners					**Model 6**	
0–1	661 (7.2)	233 (6.6)	428 (7.5)	1.00	1.00	
2–5	5384 (58.0)	2123 (60.0)	3243 (56.8)	1.20 (1.01–1.42)	1.15 (0.96–1.37)	0.129
6+	1007 (10.9)	397 (11.2)	610 (10.7)	1.20 (0.98–1.47)	1.11 (0.89–1.38)	0.298
Missing data	2215 (24.0)	787 (22.2)	1428 (25.0)	1.01 (0.84–1.21)	1.09 (0.88–1.34)	0.378
p-value (trend)				0.040	0.020	

Notes

a. The total for alcohol, smoking and contraceptive do not add up to the whole total because some of the cancers were removed from the list of controls (see Table 3 in the appedix).

b. Odds Ratios were adjusted for age, education level, marital status, period of the interview, place of residence (rural and urban).

c. The sets of controls that were used are different in each model depending on the association between the exposure and cervical cancer.

https://doi.org/10.1371/journal.pone.0260319.t002

1.25 (95% CI: 1.07–1.46) for women reporting three or more children Table 2. Compared to never smokers, the risk of cervical cancer significantly increased among women who were current smokers (OR_{adj} 1.55, 95% CI: 1.34–1.80) and ex-smokers (OR_{adj} 1.26, 95% CI: 1.02–1.55). The odds ratios for cervical cancer were 1.44 (95% CI: 1.26–1.65) for women in the age group 35–44 years and 1.33 (95% CI: 1.18–1.50) for those in the age group 45–54 years old compared to women in the age group 55–64 years. The risk of cervical cancer increased among married women compared to never married (OR_{adj} 1.14, 95% CI: 1.01–1.28). The risk of cervical cancer

Table 3. Age-adjusted p-value for hetrogeneity in prevalence of cofactors across different cancer types in controls.

	Infection unrelated cancer controls			Smoking unrelated cancer controls			Alcohol unrelated cancer controls			Hormonal contraceptive unrelated cancer controls		
Co-factors	*DF	Chi- square	p-value	DF	Chi-square	#p-value	DF	Chi-square	p-value	DF	Chi-square	p-value
Parity	-	-	-	-	-	-	-	-	-	10	9.02	0.417
HIV	9	15.2	0.076	-	-	-	-	-	-	-	-	-
Number sexual partner	9	11.02	0.274	-	-	-	-	-	-	-	-	-
Smoking	-	-	-	3	5.44	0.142	-	-	-	-	-	-
Alcohol use	-	-	-	-	-	-	8	17.13	0.029	-	-	-
Contraceptive use	-	-	-	-	-	-	-	-	-	15	30.82	0.051

*DF = degrees of freedom

#p-value <0.05

https://doi.org/10.1371/journal.pone.0260319.t003

among women who reported having consumed at least 47.1 grams of alcohol per week (heavy alcohol consumption) was 44% higher (OR_{adj} 1.44, 95% CI: 1.15–1.81) compared to women who reported having never consumed alcohol. Cases were more likely than controls to have ever used injectable contraceptives (OR_{adj} 1.34, 95% CI: 1.11–1.61) and ever used oral or injectable contraceptives (OR_{adj} 1.17, 95% CI: 1.01–1.37). A lower level of educational attainment was associated with an increased risk of cervical cancer: Education (None versus Secondary and above OR_{adj} 2.01, 95% CI: 1.73–2.34) and (Primary versus Secondary and above OR_{adj} 1.60, 95% CI: 1.44–1.77). Living in rural compared to urban areas increased the risk of cervical cancer by 62% (OR_{adj} 1.62,95% CI: 1.44–1.77).

Our estimated PAF of cervical cancer for the 7 modifiable cofactors ranged from HIV(positive) (17.6%), lower education attainment (no education or primary) (16.9%), higher parity (3+ children) (12.6%), contraceptive use (Ever injectable and/ or oral) (8.9%), alcohol consumption (light and heavy) (5.6%), smoking (ex-smoker and current smoker) (5.1%) and 4.4% for the place of residence (rural) Fig 2. The number of sexual partners were excluded as it was not statistically significant.

We tested the robustness of our control selections on the assumption that the prevalence of exposure for each of the cancer types chosen in each of the comparisons should be homogeneous. We therefore, calculated an adjusted (age, number of sexual partners and education level) Mantel-Haenszel Chi-square tests of heterogeneity for each type of comparisons. We observed no differences ($p > 0.05$) in the prevalence of cofactors across different cancer types in the three control arms except for alcohol Table 3.

Discussion

In descending order, cervical cancer was associated with being HIV-positive, educational attainment, higher parity, contraceptive use, heavy consumption of alcohol, current smoking and residing in rural areas among black South African women.

In the current study, cervical cancer risk was significantly associated with HIV infection, which was ranked as the most important risk factor, with a PAF of 17.6%. This implies that about 18% of cervical cancers would be prevented if individuals were not infected with HIV. This is particularly important in South Africa where black women are disproportionally affected by HIV compared to other racial groups [23]. The association between HIV and cervical cancer is supported by several studies in low- and middle-income countries [24, 25], showing that during HPV pathogenesis, coinfection with HIV increases HPV viral persistence [25]. Our finding is similar to the previous study on the spectrum of HIV-1 related cancers in South Africa which used the JCS data. In that study, the risk of cervical cancer was elevated in HIV-1 positive women, OR = 1.6 (95% CI: 1.1–2.3) [26]. Similarly, in a Ugandan study, HIV positive

women had an increased risk for cervical cancer (OR_{adj} = 2.4, 95% CI: 1.1–4.4) [8]. Efforts to reduce new HIV infections among black women in South Africa would likely reduce the incidence of cervical cancer in these women in future.

High incidence rates of cervical cancer have been reported among women with low socio-economic status in both high and low-income settings [27, 28]. Socio-economic status may affect cervical cancer incidence via levels of educational attainment. Education, a measure of socio-economic status, is inversely related to cervical cancer risk [29]. In a study from Spain and Columbia having no education was associated with cervical cancer (OR = 2.5, 95% CI: 1.6–3.9) [30]. Similarly, our study found having no education or completing primary school was associated with an increased risk of cervical cancer. Our results indicate that 16.9% of the cervical cancers would have been prevented if women with primary education had secondary and above education. Future investments in education should drive cervical cancer rates downwards.

In our study, higher parity (was possibly related with age at first sexual debut and possibly early exposure to HPV infection) was associated with the risk of developing cervical cancer and a PAF of 12.6%. Our findings are in agreement with Briton et al. [31], Castellsague et al. [5] and Jensen et al. [32] who demonstrated an association between higher parity (2 or more children) and cervical cancer risk. The International Agency for Research on Cancer (IARC) showed a 3.8 (95% CI: 2.7–5.5) times risk for cervical cancer with 7 or more full-term pregnancies [33]. Having 2 or more children was associated with persistent HPV infection which facilitated the development of cervical cancer [32]. Similar to our study, PAF's of between 24% and 44% were reported for the USA and Italy [34] and 42% in Costa Rica for higher parity [10]. Evidence suggests that the burden of cervical cancer should decrease if average family sizes decrease [33, 35].

Prolonged use of hormonal contraceptive has been associated with cervical cancer [36]. A previous study from the JCS demonstrated an association between the duration of hormonal contraceptive use and cervical cancer [13]. In our study, we did not consider the duration of contraceptive use and cervical cancer. Nonetheless, we reported OR = 1.48 (95% CI: 1.24–1.77) and a PAF of 8.9%. Our findings which showed an association between the use of both injectable and oral contraceptive use and cervical cancer are broadly similar to the JCS findings of Urban et al. [13], (OR = 1.38, 95% CI: 1.08–1.77), as well as for injectable only and cervical cancer (OR = 1.58, 95% CI: 1.16–2.15). Our finding is also comparable to that of Appleby et al. [36], which contains earlier JCS data, where combined contraceptive use was associated with cervical cancer. Similarly, in Latin America, Herrero et al. [37] found an association between injectable contraceptive use and cervical cancer, which reduces significantly after cessation of use. Thus, prolonged use of hormonal contraceptives should be time-limited to avoid excess risks.

The evidence concerning alcohol consumption and the risk for cervical cancer is equivocal. Some studies demonstrated an association with alcohol consumption [9, 38, 39] but others did not [40, 41]. Our study, however, found a significantly elevated risk for cervical cancer in heavy drinkers. Our data on PAF suggest that 5.6% of cervical cancer cases would be avoided if alcohol consumption were reduced. It is possible that women who were heavy drinkers in our study may have also been involved in other high-risk behaviours such as smoking, having multiple sexual partners and other behaviours that promote the acquisition of hr-HPV [42]. Notably, lack of enough data on cervical screening made it difficult to control for it, thus we can not preclude the effects of residual confounding.

Current smoking has consistently been associated with cervical cancer pathogenesis. We reported an OR of 1.55 (95% CI: 1.34–1.80) for current smokers and a PAF of 5.1% for smoking. A 2008 JCS study, found current smokers in South Africa had an increased risk of cervical

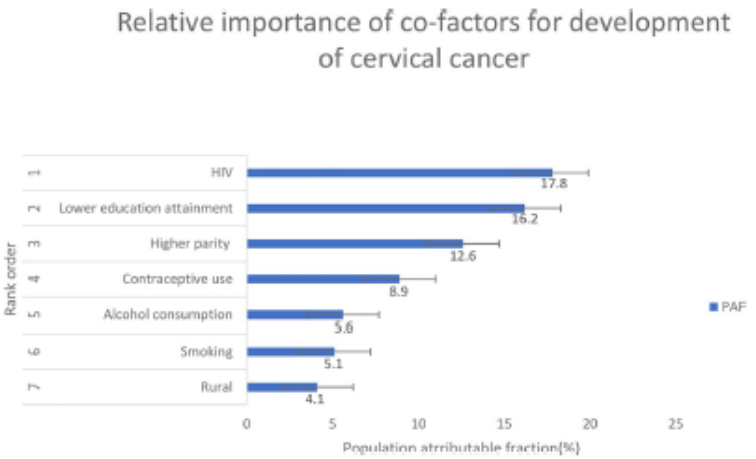

Fig 2. Population attributable fraction of the cofactors on cervical cancer.

https://doi.org/10.1371/journal.pone.0260319.g002

cancers relative to never smokers (OR = 1.5, 95% CI: 1.2–1.8) [43]. Similarly, Roula et al. [44] in a European prospective cohort study, showed current smokers had a Hazard Ratio (HR) of 1.6 (95% CI: 1.4–2.5) for cervical cancer. Besides, a 2011 international study by IARC showed that current smokers compared to never smokers had a OR = 1.94 (95% CI: 1.26–2.98) risk of developing cervical cancer [45]. While smoking is an important risk factor for cervical in other populations, it might not be as important a risk factor in black South African women, where the prevalence of smoking is low (4.1%) [46]. Continuing progressive anti-smoking laws and health promotion in South Africa is needed to retain low smoking prevalence among Black females.

We found an association between residing in the rural area and cervical cancer. Our finding is comparable to the findings of Yang et al. [47], which showed an association between rural area and cervical cancer (OR = 1.46, 95% CI: 1.03–2.06) and a PAF of 4.4%. This could be due to a lack of cervical cancer screening services, poverty in rural areas, lower education attainment and low uptake in cervical cancer screening. Vhuromu et al. [48], reported low utilization of cervical cancer screening in rural areas. Improved health systems and new methods of cervical cancer screening services in rural areas may result in an improved uptake in cervical cancer screening. Such efforts would likely reduce cervical cancer cases in rural areas.

Sexual intercourse is the main route for HPV transmission. Having multiple sexual partners increases the risk of HPV infection among women which may result in cervical cancer. Other studies have demonstrated an association between the number of sexual partners and cervical cancer risk [49, 50]. Liu et al. [49], used a meta-analysis of 41 studies and found the number of sexual partners was an independent risk factor for cervical cancer even after adjusting for HPV infection. Appleby et al. [50], in an international collaboration of 21 epidemiological studies demonstrated an association between the number of sexual partners and cervical cancer. In our study, we did not find an association between the number of sexual partners and the risk of cervical cancer. However, there was a small increase in risk which was not significant Table 2. The possible explanation for non signifanct finding in our study could be a result of small samples size among women aged 25–34, reporting bias (erroneously reporting fewer

sexual partners than in reality), confounding by HIV, and no partner data available on male partners.

Our study supports the importance given to the cofactors of cervical cancer and the need for effective interventions of these cofactors. In South Africa, cervical cancer rates could increase or decrease depending on the effectiveness of public health policies. The PAF takes into account the prevalence of the cofactor and adjusted odds ratios, underscoring that an increase in the prevalence of these cofactors could have a notable effect on cervical cancer incidence among black women in South Africa.

There are some strengths to our study. The larger sample size allowed us to estimate the contribution of key lifestyle cofactors more reliably than before. The selection of appropriate sets of cancer controls unrelated to a specific exposure of interest minimised the bias of the estimates related to the exposure, and referral, interviewer and recall biases [17, 18]. Both cases and controls were patients with cancers and they are likely to remember their past exposures in a similar way. This is the first study that attempts to measure the relative importance of a wide range of risk factors for cervical cancer in the same study population, by adjusting for a common set of confounders.

This study has several limitations. The effects obtained were not adjusted for frequency of Pap smear screening since the data available was self-reported and the question of Pap smear was only added in 2001. We could not control for unmeasured confounders despite adjusting for known confounders such as age and HIV status. Since the study only focused on black South African women, mainly resident in Johannesburg and Soweto, the findings from this study may not be generalizable to the entire population of South Africa. Another limitation of this case-control study is reverse causality whereby people with long-standing conditions may change their lifestyle. We mitigated this by reclassifying those who reported quitting smoking five years before diagnosis as current smokers, in case they altered their habits because of underlying health problems. Although the questions on alcohol consumption were focused on before the participant became ill, we could not perform a similar re-classification as for smoking. Because different exposures required different control selections, we avoided measuring combined exposures e.g. smoking and drinking.

In conclusion, in order of importance, HIV-positivity, educational attainment, parity, hormonal contraceptive use, alcohol, smoking and residing in rural area were associated with cervical cancer among black South African women. These women should be prioritized in opportunistic or planned cervical cancer screening programs. Our findings confirm previosuly known cofactors of cervical cancer and provide a rank order of risks that could be used locally to target educational messaging and appropriate interventions.

Supporting information

S1 Table. List of cancers included in each control group.
(DOCX)

S1 Data.
(XLS)

Acknowledgments

We acknoweldge all who provided feedback to improve the manuscript.

Author Contributions

Conceptualization: Mwiza Gideon Singini.

Data curation: Wenlong Carl Chen, Melitah Motlhale, Abram Bunya Kamiza, Mazvita Muchengeti.

Formal analysis: Mwiza Gideon Singini.

Funding acquisition: Freddy Sitas, Debbie Bradshaw, Cathryn M. Lewis, Christopher G. Mathew, Tim Waterboer, Robert Newton.

Investigation: Debbie Bradshaw, Christopher G. Mathew, Tim Waterboer, Robert Newton, Elvira Singh.

Methodology: Mwiza Gideon Singini.

Software: Mwiza Gideon Singini.

Supervision: Freddy Sitas, Elvira Singh.

Visualization: Wenlong Carl Chen.

Writing – original draft: Mwiza Gideon Singini.

Writing – review & editing: Mwiza Gideon Singini, Freddy Sitas, Debbie Bradshaw, Wenlong Carl Chen, Melitah Motlhale, Abram Bunya Kamiza, Chantal Babb de Villiers, Cathryn M. Lewis, Christopher G. Mathew, Tim Waterboer, Robert Newton, Mazvita Muchengeti, Elvira Singh.

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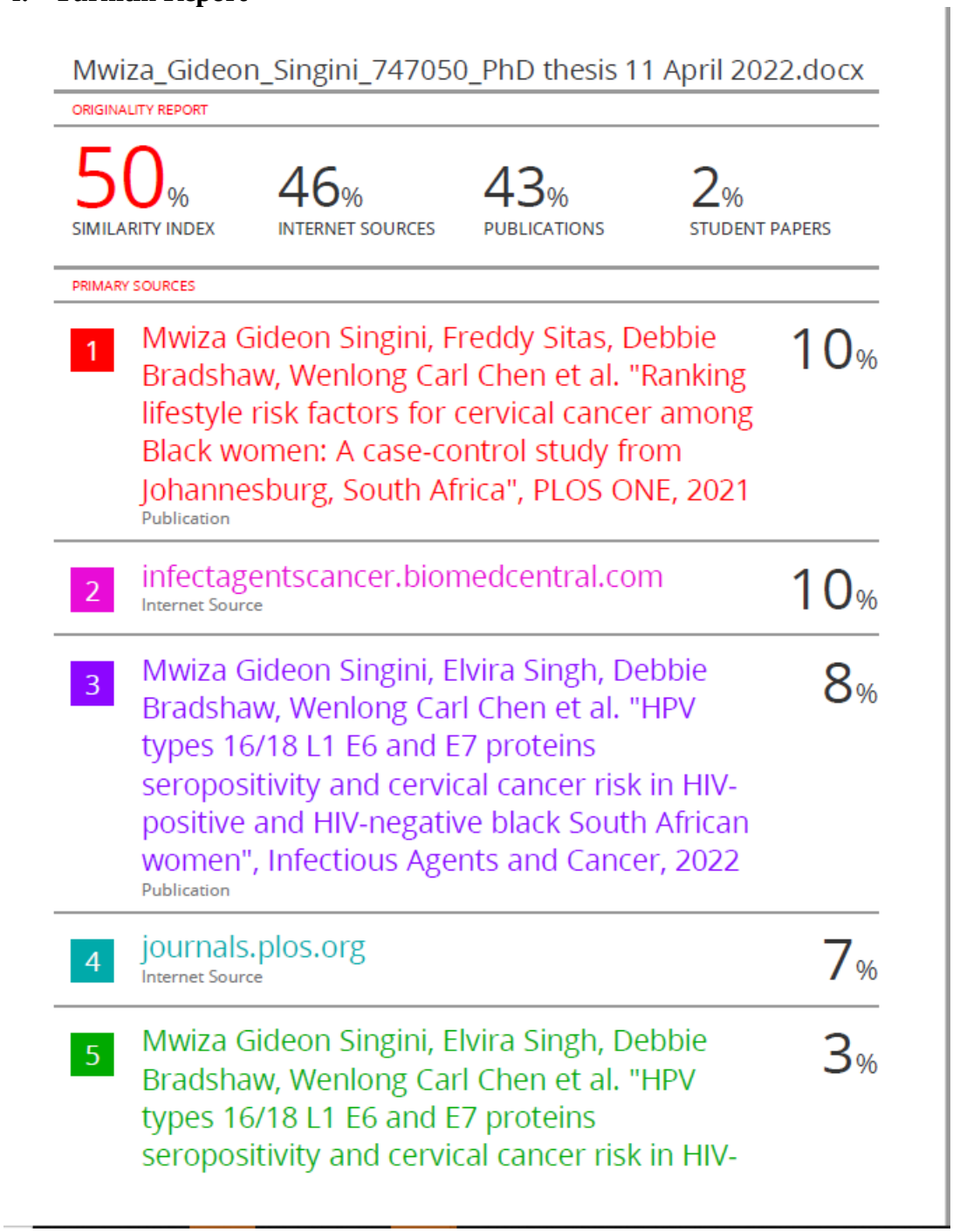
f. Turnitin Report



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ORIGINALITY REPORT

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g. Motivational letter from supervisor

NATIONAL HEALTH LABORATORY SERVICE
National Cancer Registry

National Cancer Registry
1 Modderfontein Road, Sandringham, Johannesburg 2131
Tel: +27 (0)11 885 5303

Date: 25 April 2022

Research and Postgraduate Office
Faculty of Health Sciences
University of the Witwatersrand
Phillip V Tobias Health Sciences Building
29 Princess of Wales Terrace, Parktown
Johannesburg, South Africa, 2193

RE: MGSINGINI 747050: Turn-It-In report clarification for PhD Submission

Dear Colleagues,

I write this letter in support of Mr MG Singini's PhD submission. The Turn-It-In Originality report for his thesis returned a similarity index of 50%. This is due to the inclusion of his original published manuscripts as part of the thesis submission:

1. **Singini, M.G**., Singh, E., Bradshaw, D. et al. HPV types 16/18 L1 E6 and E7 proteins seropositivity and cervical cancer risk in HIV-positive and HIV-negative black South African women. Infect Agents Cancer 17, 14 (2022). https://doi.org/10.1186/s13027-022-00418-2

2. **Singini MG**, Sitas F, Bradshaw D, Chen WC, Motlhale M, Kamiza AB, et al. (2021) Ranking lifestyle risk factors for cervical cancer among Black women: A case-control study from Johannesburg, South Africa. PLoS ONE 16(12): e0260319. https://doi.org/10.1371/journal.pone.0260319

Please refer to the Originality report attached. The top nine matches, accounting for 40% of the 50% of the similarity index, are all from his original work.

NATIONAL HEALTH LABORATORY SERVICE
National Cancer Registry

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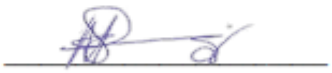
1 Modderfontein Road, Sandringham, Johannesburg 2131
Tel: +27 (0)11 885 5303

Yours sincerely,



Dr Mazvita Muchengeti
Acting Head of Department: National Cancer Registry
National Health Laboratory Service
Tel: +27(0)11 885 5303 | Mobile: +27(0)76 507 9413
MazvitaM@nicd.ac.za | www.ncr.ac.za | www.nhls.ac.za

NATIONAL HEALTH LABORATORY SERVICE
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Practice Number: 5200296